EFFECTS OF GLOBAL CHANGE ON BENTHIC COMMUNITIES OF THE LOWER EBRO RIVER: IMPLICATIONS FOR THE ASSESSMENT OF THE ECOLOGICAL STATUS

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EFFECTS OF GLOBAL CHANGE ON BENTHIC COMMUNITIES OF THE LOWER EBRO RIVER: IMPLICATIONS FOR THE ASSESSMENT OF THE ECOLOGICAL STATUS.

Ph.D. THESIS

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WE CERTIFY that the present study, entitled "EFFECTS OF GLOBAL CHANGE ON BENTHIC COMMUNITIES OF THE LOWER EBRO RIVER: IMPLICATIONS FOR THE ASSESSMENT OF THE ECOLOGICAL STATUS", presented by Luis Alberto Quevedo Báez for the award of the degree of Doctor, has been carried out under our supervision at the Aquatic Ecosystems Program of the Institute for Food and Agricultural Research and Technology (IRTA).

Ph.D. Thesis Supervisors:

Dr. Carles Ibáñez　　　　Dr. Nuno Caiola

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Abstract

This Ph.D. thesis assess the structure and composition of benthic communities (macroinvertebrates and diatoms) of the lower Ebro River as a function of alterations in the natural river flow caused by the presence of the water regulation system, and changes in the temperature regime caused by a sustained increase of water temperature (produced by the Ascó nuclear power station).

Initially, the entire hydrogeomorphic variability of the lower Ebro was considered and surveys conducted along five river sections integrating different years and seasons were analysed in order to assess spatial and temporal changes in community structure. Significant differences for macroinvertebrates were found between the section closest to reservoirs and the rest of the study sections; as well, indices for ecological status assessment based on both macroinvertebrates and diatoms showed lowest scores at this section, which is attributable to the habitat degradation caused by direct influence of the dams.

Thereafter, the influence on benthic communities (macroinvertebrates and diatoms) of an increase in temperature caused by the cooling system of the Ascó nuclear power station was assessed; for this purpose, surveys conducted at sites before and after the effluent and collected from natural and artificial substrata were analyzed. Benthic assemblages showed sensitivity to thermal changes both in natural and artificial substrata, even though warming did not exceed 3 ^{o}C. Factors that seemed to influence benthic assemblages the most were water warming caused by the nuclear power station and seasonal variation in nutrients and conductivity. Given that warming conditions in the study area have been permanent during the last 30 years, results could be useful to assess the impacts of global warming on large Mediterranean rivers.

Presentations and publications derived from the Thesis:

Quevedo, L., 2012. Efectos del Cambio Climático en comunidades bentónicas del Bajo Ebro y sus implicaciones para la evaluación del estado ecológico. “II Foro de estudiantes Ecuatorianos en Europa”. Consulado General del Ecuador en Milán, Secretaría Nacional de Educación Superior Ciencia y Tecnología (SENESCYT). Milán, Italia.

Quevedo, L., C. Ibañez, N. Caiola & R. Trobajo, 2013. Structure of benthic communities (macroinvertebrates and diatoms) in the lower stretch of a Mediterranean highly regulated large river. VII Congreso Ibérico sobre Gestión y Planificación del Agua. Fundação Calouste Gulbenkian, Fundación Nueva Cultura del Agua. Lisboa, Portugal.

Quevedo, L., C. Ibañez, N. Caiola & R. Trobajo, 2014. Structure of benthic communities (macroinvertebrates and diatoms) in the lower stretch of a Mediterranean highly regulated large river. XVII Congress of the Iberian Association of Limnology (AIL). Santander, Spain.

Quevedo, L., C. Ibañez, R. Trobajo & N. Caiola. Benthic diatom communities of a large Mediterranean river under the influence of a thermal effluent. **IN REVIEW**. Limnetica. Nº Ref.: LIMCAT 79-014. 19/12/2014.

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Quevedo, L., C. Ibañez, N. Caiola & D. Mateu. Effects of thermal pollution on benthic macroinvertebrate communities of a large Mediterranean river. **TO BE SUBMITTED**. Limnetica.

Contents

ABSTRACT -- **4**

PRESENTATIONS AND PUBLICATIONS DERIVED FROM THE THESIS: ------ 5

GENERAL INTRODUCTION-- **9**

CHAPTER 1--**31**

Benthic macroinvertebrate and diatom communities of a large, highly regulated Mediterranean river (Lower Ebro River, Catalonia, Spain)
Quevedo, L., C. Ibañez, N. Caiola, R. Trobajo, N. Cid & H. Hampel
Limnetica (In review)

CHAPTER 2--**81**

Benthic diatom communities of a large Mediterranean river under the influence of a thermal effluent
Quevedo, L., C. Ibañez, R. Trobajo & N. Caiola
Limnetica (In review)

CHAPTER 3-- **119**

Effects of thermal pollution on benthic macroinvertebrate communities of a large Mediterranean river
Quevedo, L., C. Ibañez, N. Caiola & D. Mateu
Limnetica (to be submitted)

GENERAL DISCUSSION --- **153**

General introduction

Rivers have provided resources for the advancement of human civilization from its beginnings and have been the source of water supply for human activities as agriculture, industry, power generation, transportation, domestic needs, fishing and other recreational activities (Allan & Castillo, 2007). But, the increase of human population and economic development have intensified competition over water resources worldwide, leading to predictions of even greater future conflicts over water supplies (e.g. Gleick, 1993; McCaffrey, 1993). Rivers have an essential role in maintaining human health and wellness, but during the last decades, the degradation of these aquatic ecosystems has been evidenced as a result of the overexploitation of resources (e.g., water withdraw for irrigation), the alteration of natural process (e.g., alteration of the flow regime by dams) and pollution (both organic and chemical), leading in most of cases to the impairment of their ecological status.

Damming is one of the greatest modifications of the fluvial landscape and during the last centuries several river systems throughout the world have been regulated mainly to extend urban and agricultural areas, enable or facilitate river navigation, generate power and reduce flooding risk. Approximately 800.000 dams have been constructed worldwide and on a global scale river damming has increased the mean residence time of river waters from 16 to 47 days and has increased the volume of standing water in more than 700 percent (Friedl & Wüest, 2002; Gleick, 1998; Petts & Gurnell, 2005; Skalak *et al.*, 2013). Furthermore, large rivers usually present several dams and conceptual models (Fig. 1) about the cumulative impacts of a sequence of dams on the river geomorphology and ecology have been presented (e.g. Skalak *et al.*, 2013).

These alterations have lead to functional modifications of the entire river ecosystem (Peter, 1998), through the reduction of river floodplain area and the loss of hydraulic and morphological variability (Ward *et al.*, 2001); and thereby influencing the abundance and diversity of aquatic organisms living in rivers. In fact, effects of damming on river morphology and ecological processes have become a main subject for river research and watershed management.

Thermal pollution is another important source of alteration in rivers, and often arises from the waste heat generated by industrial process such as certain power generation stations (Fig. 2). Higher water temperature usually rises the metabolic rate of aquatic organisms, which can in the end modify the balance of species composition (Langford, 1990); changes in community structure as response to thermal disturbances have been detected even with a temperature alteration of few degrees centigrade (Kaushal *et al.*, 2010). Many authors have studied the ecological consequences of thermal pollution (e.g. Caissie, 2006; de Vries *et al.*, 2008; Langford, 1990), and their results include changes in abundance and diversity of aquatic organisms (e.g. De Nicola, 1996; Gibbons & Sharitz, 1981; Wellborn & Robinson, 1996) as well as in the creation of an environment hospitable to alien species (e.g. Leuven *et al.*, 2007; Wijnhoven *et al.*, 2003). Globally, the demand for cooling in power stations and other industrial process has increased considerably, multiplying thermal pollution sources worldwide.

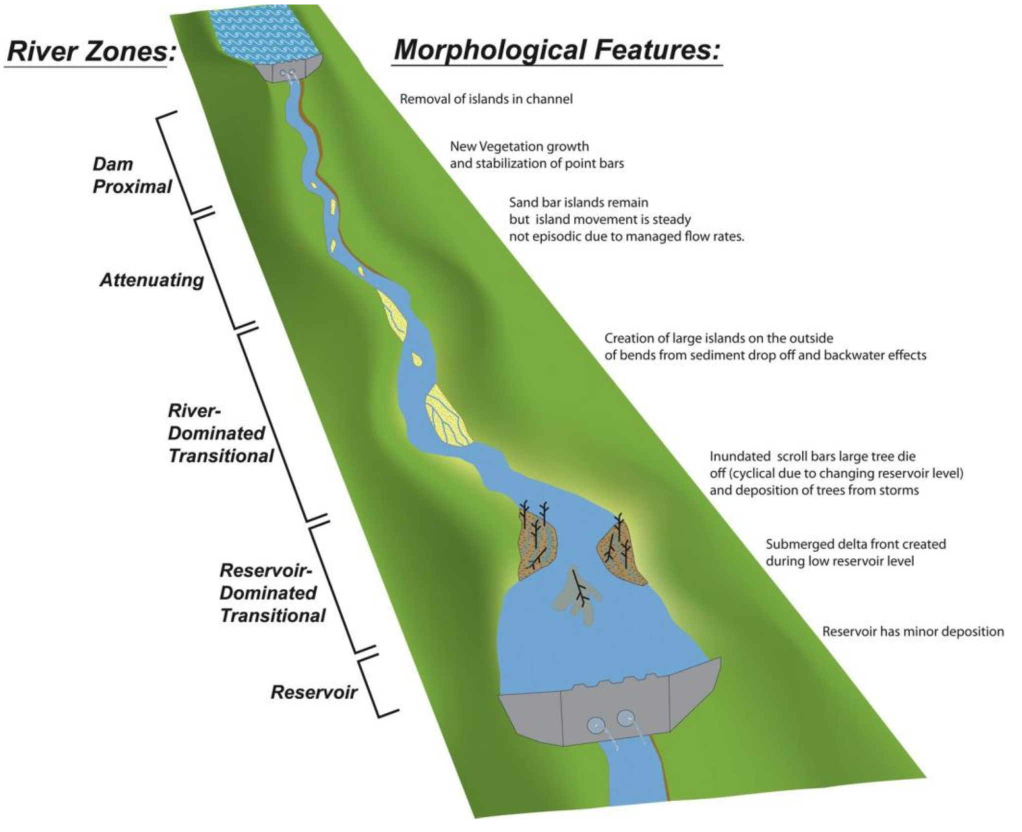

Fig. 1 Conceptual model of channel morphology that results from dam interaction along a river reach. Extracted from Skalak *et al* (2013).

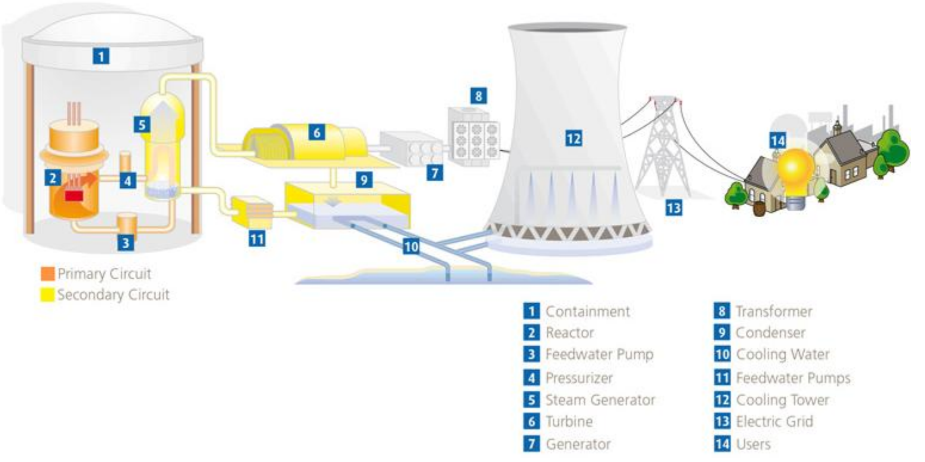

Fig. 2 Functional diagram of a nuclear power station using river water to its cooling system. Extracted from (http://www.anav.es)

In large rivers, the degradation is more notorious at the lower stretches due to higher cumulative pressures that are often the cause of the greatest sustained environmental impacts. Ecological interactions at the lower river section can be severely affected by the presence and management of large dams located upstream with agricultural, industrial and recreational proposes; and by their collateral implications (e.g. thermal pollution and alteration of sediment transport). Since aquatic life is conditioned by the timing and quantity of river flow, upstream disruptions can be the cause of modifications of the structure of biological communities, often becoming more severe at the lower stretch of large rivers.

In addition, rivers located in the Mediterranean region, exhibit unpredictable and high year-to-year variability in precipitation resulting in lengthy periods of drought and devastating floods. This wide natural climatic variability combined with anthropogenic pressures as damming and water extraction can make Mediterranean watersheds particularly vulnerable (Sabater & Tockner, 2010). In fact, Mediterranean watersheds are among the most heavily impacted by anthropogenic activities (Liquete *et al.*, 2009), and these impacts range from local scale (e.g. thermal effluent discharges, modification of river hydromorphology) to global scale (e.g. climate change impacts) (Caissie, 2006).

The IPCC (2007, 2014) states that the Mediterranean region is highly vulnerable to human induced climate changes and may be one of the most affected by global warming. Climatic patterns will change under the influence of anthropogenic pressures (IPCC, 2014), stressing the natural fluctuations and altering the hydrological cycle of aquatic ecosystems. Inter-annual variability is projected to increase in the Mediterranean region, and temperature as well as the occurrence of droughts are predicted to increase within the present century (Giorgi & Lionello, 2008). For these reasons, the Mediterranean climate zone is considered a hotspot in future climate change projections (Giorgi & Lionello, 2008).According with these projections, climate patterns and their influence (direct or indirect) on other environmental parameters such as food resources and water temperature, will determine the structure and function of biological communities in Mediterranean rivers (Álvarez & Pardo, 2009; García *et al.*, 2008; Langton & Casas, 1999).

Therefore, different biological groups have often been used to assess environmental impacts caused by different stressors, and benthic communities (diatoms and macroinvertebrates) are widely utilized as bioindicators of ecological status. Biomonitoring protocols based on these biological groups are used for running waters worldwide (e.g. Alba Tercedor *et al.*, 2002; Furse, 2006; Kelly & Whitton, 1998; Metcalfe, 1989; Quevauviller *et al.*, 2008). Moreover, by considering these two biological groups, both long-term and short-term changes of environmental conditions can be detected (Li *et al.*, 2010).

Diatoms have an important function as primary producers in river ecosystems (Lamberti, 1996; Stevenson *et al.*, 1996), and are involved in different biogeochemical cycles (e.g. nitrogen, carbon and silica cycling) (Mann, 1999; Thornton *et al.*, 2002), responding strongly and quickly to environmental changes (Round, 1991; Round *et al.*, 1990). Diatoms are valuable indicators of environmental conditions in rivers (Smol & Stoermer, 2010; Whitton & Kelly, 1995) and have been preferred for river biomonitoring purposes by many authors (e.g. Chessman *et al.*, 1999; McCormick & Cairns Jr, 1994; Whitton *et al.*, 1991). Several biotic indices have been successfully applied to estimate the status of river ecosystems (e.g. Eloranta & Soininen, 2002; Goma *et al.*, 2005; Kelly *et al.*, 2009; Prygiel & Coste, 1993).

Macroinvertebrates are also key components of aquatic food webs that link organic matter and nutrient resources (e.g., leaf litter, algae and detritus) with higher trophic levels (Wallace & Webster, 1996). With a sensitive life stage and relatively long life span, they have the ability to integrate the effects of environmental variations allowing the analysis of responses to environmental stress (Basset *et al.*, 2004; Rosenberg & Resh, 1993). The potential of benthic macroinvertebrates as bioindicators for aquatic ecosystems has been widely reported (e.g. Buffagni *et al.*, 2004; Rosenberg & Resh, 1993; Statzner *et al.*, 2001) including its use in the assessment of anthropogenic hydrological alterations (Dunbar *et al.*, 2010; Gore *et al.*, 2001; Suren & Jowett, 2006; Vivas *et al.*, 2002). Furthermore, macroinvertebrate and diatom assemblages have been used contemporaneously for monitoring river ecosystems (Johnson *et al.*, 2006; Soininen & Könönen, 2004; Torrisi *et al.*, 2010).

Study context: the lower Ebro River

The Ebro is the only large river in the Iberian Peninsula that flows into the Mediterranean Sea (Barceló & Petrovic, 2011). It is located in the NE of the Iberian Peninsula and with a surface of 85 534 km^2 is one of the most important tributaries to the Mediterranean Sea and one of the largest catchments in Europe (Tockner *et al.*, 2009). The main river is 928 km long and its principal tributaries are the Segre, Aragón, Cinca, and Gállego rivers. The climate is continental in most of the basin with a transition from mountain climate at the north (Pyrenees) to Mediterranean climate at the lower part.

The population density in the Ebro territory (33 people/km^2) is lower than the Spanish mean (78 people/km^2), and there are a total of 2.800.000 people inhabiting mainly in the middle course of the river (www.chebro.es).The dominant land use of the Ebro basin is agriculture (Grantham *et al.*, 2013), but it has been also affected by numerous impacts derived from industry and urban uses (Lacorte *et al.*, 2006; Pujol & Sánchez-Cabeza, 2000; Terrado *et al.*, 2006).

The river is strongly regulated by nearly 190 dams (Batalla *et al.*, 2004) which impound more than the 60% of the mean annual runoff for irrigation purposes and hydropower generation (Vericat & Batalla, 2006); approximately 90% of the water is used for irrigating more than 1 million hectares (Ibáñez *et al.*, 2008).The mean annual flow near the upper end of the estuary (Tortosa) was 592 m^3/s at the beginning of the 20th century but, increasing water uses has led to a decreasing tendency since the 70's, down to about 400 m^3/s (Ibáñez *et al.*, 1996).

The studies reported in this Thesis were carried out in the lower Ebro River (Fig. 3) (100 km upstream the mouth). In this part of the Ebro there are two large reservoirs, Mequinensa (Fig. 4) and Riba-roja (Fig. 5), built in the 1960s for hydropower purposes. The river hydrology, geomorphology and ecology are strongly impacted by the existence, features and operation of these reservoirs. Downstream it is located a smaller reservoir at Flix town, with a capacity of 11.4 hm^3.

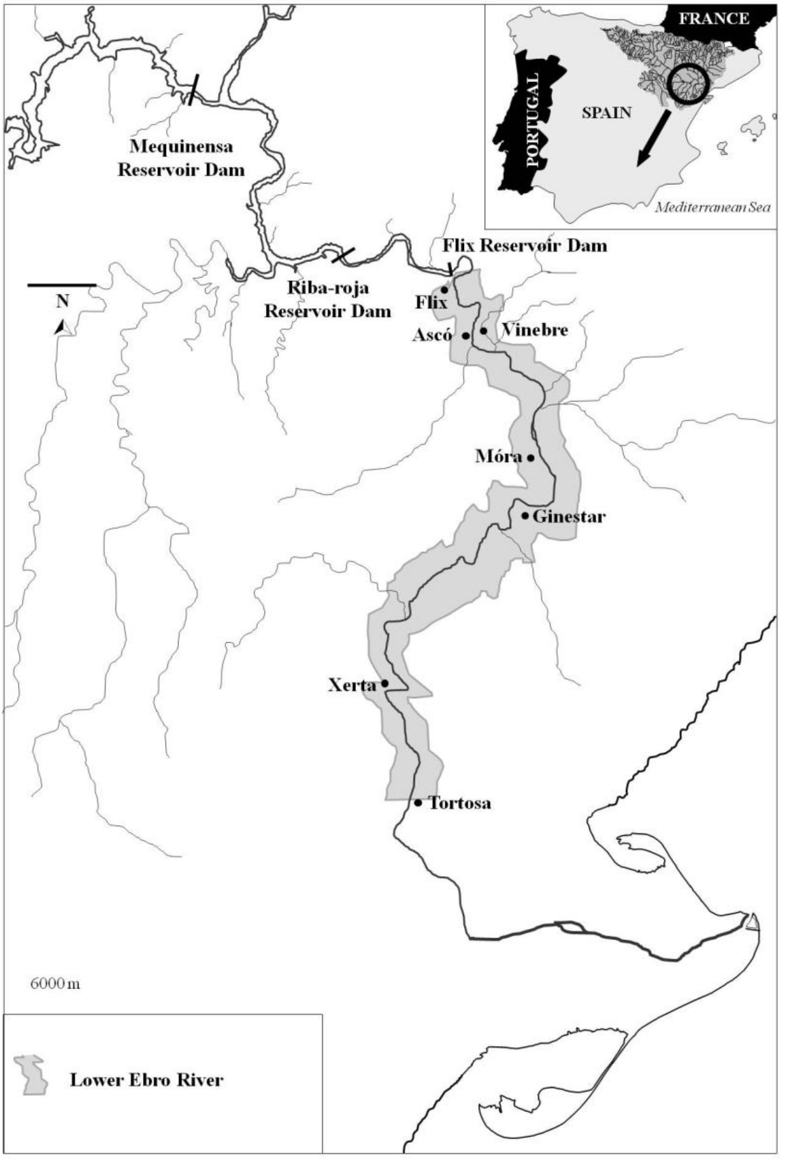

Fig. 3 Map of the lower Ebro River, showing the study area (grey shading).

Fig. 4 Mequinensa dam. Extracted from (http://www.saihebro.com)

Fig. 5 Riba-roja dam. Extracted from (http://www.saihebro.com)

The water temperature regime has been altered by the presence of the Ascó nuclear power station (Fig. 6), which is located at 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. The power station was built in 1984 and has two reactors with a gross 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 granted 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 (Prats *et al.*, 2010).

Fig. 6 Ascó nuclear power station.

Consequently, the main anthropogenic factors exerting pressure on the study area are: the flow regulation system caused by the presence of the dams and the water warming caused by the nuclear power station.

Although benthic communities are indicators of the ecological status and widely used in biomonitoring programs, it is difficult to discriminate between effects from different stressors. Studies are scarce and most of the existing works have been performed in streams in part due to the difficulty in sampling benthic communities of large rivers; these studies often do not include the analysis of both macroinvertebrate and diatom communities.

In the Ebro River, benthic communities have been used to analyse the effects of chemical pollution and eutrophication (Cid *et al.*, 2010; Muñoz & Prat, 1996; Tornés *et al.*, 2007) and to assess the ecological status of the river (Oscoz *et al.*, 2007), but in the lower Ebro most of the ecological studies have focused on the estuary area (Muñoz & Prat, 1994; Nebra *et al.*, 2011; Rovira *et al.*, 2009; Rovira *et al.*, 2012a; Rovira *et al.*, 2012b).

The Catalan Water Agency (ACA) and the Hydrographical Confederacy of the Ebro (CHE) have developed periodical monitoring programs based on benthic communities (available in http://www.chebro.es and http://aca-web.gencat.cat) to determine water quality and to assess the ecological status; however, these reports do not include information neither about community structure nor the response of the communities to anthropogenic pressures (e.g. water regulation system and the thermal increase); furthermore, most of the monitoring points are located upstream of Mequinensa and Riba-roja dams, even though, the lower part of the river evidences in great measure the consequences of the factors exerting pressure upstream.

This study includes a complete characterization of benthic communities (macroinvertebrates and diatoms) at the lower Ebro River using a methodology that covers the most relevant hydrogeomorphic variability at reach scale. However, as benthic communities are adapted to variable conditions, it is difficult to discriminate between the effects of natural and human induced stressors on the community structure. But, the presence of the Ascó nuclear power station which has been subject the river to a sustained heating during the last 30 years, provides an excellent opportunity for assessing the long term effects of warming on macroinvertebrates and diatoms. Moreover, as the range of temperature in the study area is within the bounds of climate change predictions for Mediterranean region, this becomes a "natural laboratory" suitable to predict global warming impacts on benthic communities and, therefore, on the ecological status of Mediterranean Rivers**.**

OBJECTIVES

The present Thesis was carried out in the lower course of the Ebro River; firstly, considering the entire hydrogeomorphic variability of the lower Ebro at reach scale and identifying the structure and distribution patterns of benthic communities as function of anthropogenic alterations; and afterwards, assessing the long-term effects of water warming on benthic communities (macroinvertebrates and diatoms).

Main goal:

The main goal of this study was to assess the influence of anthropogenic pressures (flow regulation system and thermal increase) on benthic communities inhabiting the lower Ebro River.

For this purpose, this thesis is organized as compendium of three chapters, corresponding to three article manuscripts to be published in SCI journals. In each manuscript some of the specific objectives were achieved and globally the manuscripts achieved the main goal.

Specific Objectives:

- To characterize the community structure of benthic diatoms. (Chapter 1 and 2)
- To characterize the community structure of benthic macroinvertebrates. (Chapter 1 and 3)
- To identify spatial patterns of benthic communities (diatoms and macroinvertebrates). (Chapter 1)
- To assess the influence of the flow regulation system and other stressors on benthic communities (diatoms and macroinvertebrates) (Chapter 1).
- To assess the effects of water warming on benthic diatom communities. (Chapter 2)
- To assess the effects of water warming on benthic macroinvertebrate communities (Chapter 3)
- To examine the implications related with ecological status and global warming. (Chapter 1, 2 and 3)

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Chapter 1

Benthic macroinvertebrate and diatom communities of a large, highly regulated Mediterranean river (Lower Ebro River, Catalonia, Spain)

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***Limnetica* (In review)**

Benthic macroinvertebrate and diatom communities of a large, highly regulated Mediterranean river (Lower Ebro River, Catalonia, Spain)

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Key words: benthos, large Mediterranean river, damming, ecological status, Water Framework Directive

ABSTRACT

This study aimed to examine community structure and distribution patterns of benthic macroinvertebrates and diatoms in the lower Ebro River as a function of anthropogenic alterations and considering the entire hydrogeomorphic variability.

Surveys conducted along five river sections integrating different years and seasons were analyzed and, Non-metrical Multidimensional Scaling (MDS), Similarity Percentage Analysis (SIMPER) and 1-way Analysis of Similarities (ANOSIM) were performed to assess spatial and temporal changes in community structure. The relationship between biological and environmental data was investigated with BIOENV routine and these patterns of association were illustrated through a Principal Components Analysis (PCA).

Significant differences for macroinvertebrates were found between the section closest to reservoirs and the rest of the study sections; as well, indices for ecological status assessment based on both macroinvertebrates and diatoms showed lowest scores at this section, which is attributable to the habitat degradation caused by direct influence of the dams.

In agreement with studies in other rivers, macroinvertebrates and diatoms responded rather differently to anthropogenic stressors; macroinvertebrates were more sensitive to physical changes in river habitat, while diatoms were more sensitive to water quality alterations.

INTRODUCTION

Mediterranean climate regions are hotspots of biodiversity widespread in all continents and supporting similar types of ecosystems characterized by strong spatial, seasonal and year to year variation (Kondolf *et al.*, 2012; Stamou *et al.*, 2004). Some of these regions share as a common factor the presence of large Mediterranean rivers (e.g. Ebro in Spain, San Joaquin and Sacramento in USA, Biobío in Chile) with comparable structural and functional features and similarly influenced by climatic and geomorphic settings (Fisher, 1995; Gasith & Resh, 1999; Gushing *et al.*, 1995; King *et al.*, 1988; Puckridge *et al.*, 1998).

Large Mediterranean rivers have been extensively dammed during the last century and reservoirs have been built to regulate the variability in water supplies for agricultural irrigation and power generation (Kondolf *et al.*, 2012), in fact they are considered to be subject to perhaps the highest levels of water infrastructure development in the world (Grantham *et al.*, 2013). However, while streams are the most studied component of Mediterranean (and world) rivers, there is fewer research involving large rivers due to the required sampling effort, economic cost of research, methodological problems for sampling and scarcity of this type of rivers.

Biomonitoring protocols based on benthic communities are widely used for running waters worldwide (Furse, 2006; Kelly & Whitton, 1998; Metcalfe, 1989; Quevauviller *et al.*, 2008) and, benthic macroinvertebrates and diatoms are frequently used as bioindicators of the ecological status. By analyzing these two biological groups, both long-term and short-term changes of environmental conditions can be detected (Li *et al.*, 2010).

Diatoms have been preferred for river biomonitoring purposes by many authors (Chessman *et al.*, 1999; McCormick & Cairns Jr, 1994; Whitton *et al.*, 1991), and several biotic indices have been successfully applied to estimate the status of river ecosystems (Eloranta & Soininen, 2002; Goma *et al.*, 2005; Kelly *et al.*, 2009; Prygiel & Coste, 1993). Macroinvertebrates also have been widely reported as bioindicators for aquatic ecosystems (Buffagni *et al.*, 2004; Lafont, 2011; Rosenberg & Resh, 1993; Statzner *et al.*, 2001; Vivas *et al.*, 2002) as well as indicators for the assessment of anthropogenic hydrological alterations (Dunbar *et al.*, 2010; Gore *et al.*, 2001; Lafont *et al.*, 2010; Suren & Jowett, 2006; Vivas *et al.*, 2002). Furthermore, in several studies (Johnson *et al.*, 2006; Soininen & Könönen, 2004; Torrisi *et al.*, 2010), macroinvertebrate and diatom communities have been used together comparing different assemblage responses to different anthropogenic pressures.

The European Union has led efforts to incorporate protection to these aquatic ecosystems through the expedition of the Water Framework Directive WFD 2000/60/EC (European Commission, 2000) which commits to state members to achieve a good ecological status of water bodies. One of the criteria established for this purpose is the implementation of biomonitoring programs based on benthic communities (and other bioindicators) as central elements for ecological quality assessment.

The Ebro is the largest river in Spain in terms of water discharge, and agriculture is the dominant land use of its basin (Grantham *et al.*, 2013). The lower part is regulated by a system of three reservoirs (Mequinensa, Riba-roja and Flix) which heavily modified the river hydrology, geomorphology and ecology by altering the magnitude, the timing and duration of flows, the sediment dynamics, the water temperature regime and the geochemistry (Ibáñez *et al.*, 1996). In the Ebro River, benthic communities have been used to analyze the effects of chemical pollution and eutrophication (Cid *et al.*, 2010; Muñoz & Prat, 1996; Tornés *et al.*, 2007) and to assess the ecological status of the river (Oscoz *et al.*, 2007), but in the lower Ebro most of the ecological studies have focused on the estuary area (Nebra *et al.*, 2011; Rovira *et al.*, 2009; Rovira *et al.*, 2012a; Rovira *et al.*, 2012b).

The Catalan Water Agency (ACA) and the Hydrographical Confederacy of the Ebro (CHE) have developed periodical monitoring programs based on benthic communities (available in http://www.chebro.es and http://aca-web.gencat.cat) to determine water quality and to assess the ecological status; however, these reports do not include information neither about community structure nor the response of the communities to anthropogenic pressures (e.g. water regulation system); furthermore, most of the monitoring points are located upstream of Mequinensa and Riba-roja dams, even though, the lower part of the river evidence in great measure the consequences of the factors exerting pressure upstream.

This study aimed to examine community structure and distribution patterns along spatial and temporal scales of benthic macroinvertebrates and diatoms in the lower Ebro, and to investigate the factors influencing these communities, in order to better understand the ecological organization and functioning of large Mediterranean rivers as basis for predicting the effects of global and local anthropogenic changes.

MATERIALS AND METHODS

Study area

The Ebro basin is located in the NE of the Iberian Peninsula (Fig. 1); with a surface of 85 534 km^2 it is one of the most important tributaries to the Mediterranean Sea. The main river is 928 km long and its principal tributaries are the Segre, Aragón, Cinca, and Gállego rivers. The climate is continental in most of the basin with a transition from mountain climate at the north (Pyrenees) to Mediterranean climate at the lower part. The basin has been strongly regulated by nearly 190 dams (Batalla *et al.*, 2004) and the main land use is agriculture, which accounts for approximately 90% of water usage for irrigating more than 1 million hectares (Ibáñez *et al.*, 2008). The mean annual flow near the upper end of the estuary (Tortosa) was 592 m^3/s at the beginning of the 20th century but, increasing water uses has led to a decreasing tendency since the 70's, down to about 400 $m^3\ s^{-1}$ (Ibáñez *et al.*, 1996). In the lower Ebro there are two large reservoirs, (Mequinensa and Riba-roja) built in 1964 and 1969 respectively for hydropower purposes (Ibáñez *et al.*, 1996); downstream Riba-roja, a small reservoir (Flix) and a nuclear power station (Ascó, operating since 1984) are located. A concession of 72.3 m^3/s of the Ebro's flow is granted to the power station for the cooling system, that returns water 2-3 ºC warmer (Prats *et al.*, 2010).

This study was performed in an area (Fig. 1) that extends from the reservoir furthest downstream to the upper limit of the estuary (Tortosa) where the river is about 80 km long, 150 m wide, 5 m deep and the substrate is dominated by gravels.

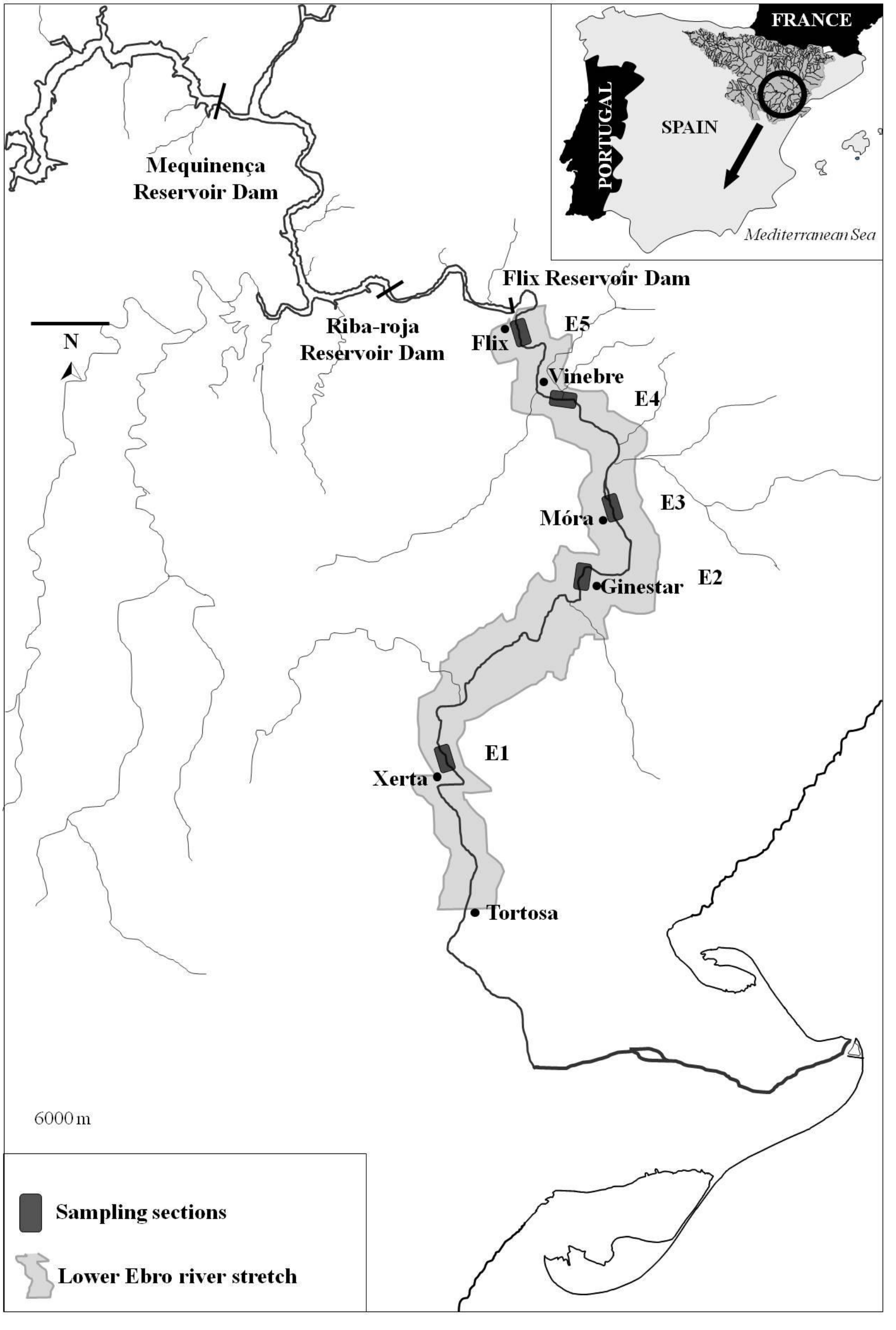

Fig. 1 Map of the lower Ebro River showing the study area and the location of sampling sections

Sampling Sites

Five (E1 to E5) sampling sections (Fig. 1) were randomly selected to perform the surveys in order to cover all the lower Ebro hydrogeomorphic variability. For this purpose aerial photographs were analyzed in order to classify the lower Ebro River in different morphological types according to a simplified classification (Rosgen, 1994). Four morphological river types were identified in the study area: steep stretches; moderately entrenched stretches; entrenched meanders; low gradient meanders. Then, a polyline GIS layer representing the lower Ebro River (available online from the Ebro Water Authority) was classified according to these four morphological river types and transformed into a point GIS layer, with equidistant points (1 Km). Thus, the aforementioned 5 sampling sections were selected in order to ensure the coverage of the morphological variability.

Benthic surveys integrating different years and seasons were conducted between 2006 and 2009, resulting in a total of 31 and 20 sampling occasions for macroinvertebrates and diatoms, respectively. Unfortunately, and for logistic reasons, diatoms were not sampled on every occasion that macroinvertebrates were collected, and never in winter. For every sampling site and occasion, physicochemical data and hydromorphological characteristics were recorded. Current velocity at 60% of total water depth was recorded with a Braystoke BFM 001 current meter; an YSI 556 multi-parameter probe was used to measure water temperature (°C), dissolved oxygen (mg/l), oxygen saturation (%), pH, salinity (ppt) and conductivity (mS/cm). Water depth (m) was measured using a Speedtech SM-5 depth-meter sounder. Substratum composition analysis was based on Wentworth (1922) scale according to the following fractions: sand (< 2 mm), gravel (2-16 mm), pebble (16-64 mm), cobble (64-256 mm), boulder (>256 mm). Analysis of total dissolved nitrogen (TDN), soluble reactive phosphorus (SRP) and SiO_4 were measured according to Koroleff (1977); and the total chlorophyll concentration was calculated using the colorimetric method (Jeffrey & Humphrey, 1975).

Macroinvertebrate sampling

Benthic macroinvertebrates were collected in the littoral zone; the riverbed was disturbed and organisms were captured using a Surber net with a mesh size of 500 µm and preserved in 4% formaldehyde; each sample was composed by fauna collected in at least three locations along each sampling section. At the laboratory, samples were rinsed in a 500 µm screen to remove fine sediments, and large organic material (whole leaves, twigs, algal and macrophyte mats) was thoroughly rinsed, visually inspected, and discarded. Then, the sample was evenly distributed in a flat pan of 30x36 cm marked with a numbered grid pattern of 6x6 cm and a random number table was used to select six squares (out of a total of 30) to ensure a representative subsample; the content of each selected square was removed to a Petri dish, sorted and identified under an apochromatic corrected stereomicroscope Leica M165C with 16.5:1 zoom and maximum 906 lp/mm. Whenever 200 organisms were not found, another square was added to the subsample.

Macroinvertebrates were identified according to Müller-Liebenau (1969), Nocentini and delle Ricerche (1985), Rossaro (1982), Tachet *et al*. (2000) and Vieira (2000) at genus level, except for Oligochaeta which were kept at sub class level and some Diptera which were kept at sub family or tribe level. Each species was classified into feeding guilds based on Tachet *et al*. (2000); the feeding guilds included: Absorber (A), Deposit feeder (D), Shredder (Sh), Scraper (Sc), Filter-feeder (F), Piercer (P), Predator (Pr) and Parasite (Ps).

Diatom sampling

Benthic diatom samples were collected from submerged natural substrata (stones) by brushing their top surfaces according to the recommendations of Kelly *et al*. (1998); each sample was a composite drawn from at least three stones. The suspension was fixed in 4% formaldehyde solution. At the laboratory, benthic samples were oxidized with H_2O_2 30% v/v for several hours in order to remove the organic matter. HCl^- 37% v/v was added to evaporate the carbonates from the samples, as described in Renberg (1990). Clean valves were permanently mounted with Naphrax© (refractive index 1.74). The permanent slides were examined using a LEICA DMI 3000 B light microscope equipped with differential interference contrast (DIC) with a 100 times oil immersion objective (n.a = 1.40). For each sampling occasion the samples collected from each river section were processed and a minimum of 400 valves counted each time. Identification of diatoms was done to species level mainly following Krammer and Lange-Bertalot (1986-1991) but other taxonomic and floristic works were also used when needed.

Data analysis

For both biological groups (macroinvertebrates and diatoms), descriptive community parameters were calculated for each river section: Richness (S), Shannon-Wiener's diversity index (H', as $\log_2$) and Pielou's evenness index (J'). Ecological status was estimated by the official indices used in Mediterranean rivers in Spain according to the WFD; these were IBMWP (Iberian Biological Monitoring Working Party) (Alba-Tercedor & Sánchez-Ortega, 1988) for macroinvertebrates and IPS (Specific Polluosensitivity Index) for diatoms calculated with the software OMNIDIA (Lecointe *et al*., 1993).

To avoid the effect of rare species, only species with a relative abundance higher than 0.05% for diatoms and 0.02% for macroinvertebrates were included in the analysis; then, abundance data was square-root transformed in order to downweight the contribution of the most abundant taxa and similarity matrices were computed using the Bray-Curtis coefficient (Legendre & Legendre, 1998). All environmental variables that expressed concentration were log-transformed and statistical analyses were performed using the different routines available in the Multivariate Ecological Research Software Package PRIMER V6 (Clarke & Gorley, 2006).

For diatoms and macroinvertebrates separately, the samples and taxon abundances were ordered using Non-metrical Multidimensional Scaling (MDS) and significant differences in assemblages composition among sections and seasons 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. Then, in order to identify resemblances between sample groups and to identify taxa that contributed to dissimilarity among sections, a Similarity Percentage Analysis (SIMPER) was performed.

The relationship between the community structure and environmental variables was investigated with the BIOENV routine (Clarke & Ainsworth, 1993; Clarke & Warwick, 2001), which maximizes a rank correlation (Spearman's coefficient) between resemblance matrices derived from biotic and environmental data, iterating for all possible combinations of environmental variables. A Spearman's coefficient value close to 0 indicates a weak relation between the community and environmental variables whereas, a value close to 1 indicates that the environmental variables selected explain the community structure. Finally, in order to illustrate patterns of association among the limnological variables identified with the BIOENV routine, two Principal

Component Analyses (PCA) were carried out with the environmental data of each biological group (because the sampling occasions were not fully coincident).

RESULTS

Physicochemical and hydromorphological parameters

The average values for water physicochemical and hydromorphological parameters measured at each sampling section are shown in Table 1. During the study period temperature ranged between 9.7 °C (E5, winter) and 24.8°C (E3, summer), and was lower in the uppermost section (E5) which is located upstream the Ascó nuclear power station and close to the Flix dam; dissolved oxygen showed highest values in spring (E3, 104%) and lowest in summer (E5, 80%) in the uppermost section; pH showed the highest and lowest values in summer (E4, 8.42; E5, 7.93); conductivity showed a minimum in spring (E4, 674 μS/cm) and a maximum in autumn (E4, 1474 μS/cm); total dissolved nitrogen showed a maximum value of 3.09 mg/l (E5, winter) and a minimum of 1.33 mg/l (E2, summer); soluble reactive phosphorus showed a minimum of 0.01 mg/l (E5, spring) and a maximum of 0.11 mg/l (E4, winter); SiO_4 ranged from 0.03 mg/l (E1, winter) to 0.87 mg/l (E1, spring); water chlorophyll varied from 0.45 μg/l (E2, spring) to 2.03 μg/l (E5, autumn). E5 section showed coarsest substrata composition (44% cobble) in relation with sections downstream (E1 E2 E3 E4), where pebbles where the dominant fraction.

Table 1. Values of physicochemical parameters measured at each sampling section. Dist= distance to the dam, T=temperature, DO = dissolved oxygen, Cond = conductivity, Sal= salinity, SPR = soluble reactive phosphate, TDN = total dissolved nitrogen, TN = total nitrogen, Chl a = chlorophyll a).

	Dist (m)	T (°C)	pH	DO (mg/l)	DO (%)	Cond (mS/cm)	Sal (ppt)	SRP (mg/l)	TDN (mg/l)	SiO_4 (mg/l)	Chl a (µg/l)	Depth (m)	Velocity (m/s)	Cobble (%)	Pebble (%)	Gravel (%)	Sand (%)
Spring																	
E1	59329.03	17.55	8.01	9.51	99.85	894.30	0.53	0.02	2.31	0.87	0.69	0.85	0.34	00	67	33	00
E2	35061.43	19.18	8.00	9.09	98.63	704.33	0.39	0.02	1.96	0.68	0.45	0.97	0.34	12	74	14	00
E3	23998.65	18.06	7.99	9.82	104.00	873.60	0.51	0.02	1.81	0.80	0.73	0.87	0.28	10	48	30	12
E4	17575.58	20.37	8.06	8.80	97.73	674.00	0.36	0.02	1.53	0.63	1.08	0.94	0.03	03	46	35	16
E5	7858.15	15.39	7.93	8.73	87.78	818.02	0.51	0.01	2.38	0.75	1.46	0.66	0.17	44	39	17	00
Summer																	
E1	59329.03	24.60	8.19	7.27	87.78	978.55	0.49	0.05	1.62	0.67	1.20	0.73	0.36	00	67	33	00
E2	35061.43	25.24	8.32	6.70	81.80	1341.50	0.67	0.03	1.33	0.75	1.07	0.97	0.34	12	74	14	00
E3	23998.65	24.85	8.06	6.82	82.46	975.68	0.49	0.04	1.55	0.64	0.84	0.80	0.38	10	48	30	12
E4	17575.58	25.73	8.42	7.62	93.90	1348.90	0.66	0.02	1.35	0.71	1.06	0.94	0.03	03	46	35	16
E5	7858.15	22.56	7.93	6.88	80.24	943.41	0.49	0.04	1.68	0.66	1.34	0.65	0.18	44	39	17	00
Autumn																	
E1	59329.03	21.50	8.33	7.85	89.30	1456.33	0.79	0.03	1.91	0.53	1.07	1.03	0.31	00	67	33	00
E2	35061.43	22.58	8.34	9.08	105.43	1474.00	0.78	0.04	2.46	0.39	0.59	0.97	0.34	12	74	14	00
E3	23998.65	22.21	8.23	7.88	90.90	1463.33	0.78	0.04	2.5	0.52	0.88	1.02	0.35	10	48	30	12
E4	17575.58	22.58	8.34	9.08	105.43	1474.00	0.78	0.04	2.49	0.55	1.45	0.94	0.03	03	46	35	16
E5	7858.15	21.50	8.33	7.85	89.30	1456.33	0.79	0.04	2.53	0.44	2.03	0.66	0.17	44	39	17	00
Winter																	
E1	59329.03	12.74	8.18	10.05	95.33	995.00	0.51	0.09	2.46	0.03	0.80	1.03	0.31	00	67	33	00
E2	35061.43	12.31	8.19	9.88	92.07	1006.50	0.52	0.08	2.96	0.06	0.78	0.97	0.34	12	74	14	00
E3	23998.65	12.30	8.19	10.14	95.39	1006.50	0.52	0.04	2.89	0.13	1.01	1.02	0.35	10	48	30	12
E4	17575.58	11.60	8.16	10.23	94.75	1006.50	0.52	0.11	3.19	0.06	0.86	0.94	0.03	03	46	35	16
E5	7858.15	9.75	8.12	10.56	93.69	1018.00	0.52	0.04	3.09	0.09	0.60	0.66	0.17	44	39	17	00

Macroinvertebrate assemblages

During the sampling period a total of 66430 individuals were collected belonging to 46 different taxa that comprised 37 genus, 36 families, 20 orders, 8 classes and 6 phyla (Appendix 1). Artropoda was the dominant phylum and accounted for 67.78% of the total abundance. Mollusca and Anellida contributed with 11.44% and 11.32% respectively. Chironomidae (28.19%), Gammaridae (15.72%) and Baetidae (9.06%) were the most abundant families. Most of the taxa found belong to Insecta and includes: 6 mayflies (Ephemeroptera), 7 caddisflies (Trichoptera), 2 Coleoptera, 2 Odonata, 10 Diptera, 1 Neuroptera and 1 Hemiptera. Macroinvertebrate community structure in each section, including a comparison with previous data available for E1, E4 and E5 is shown in figure 2.

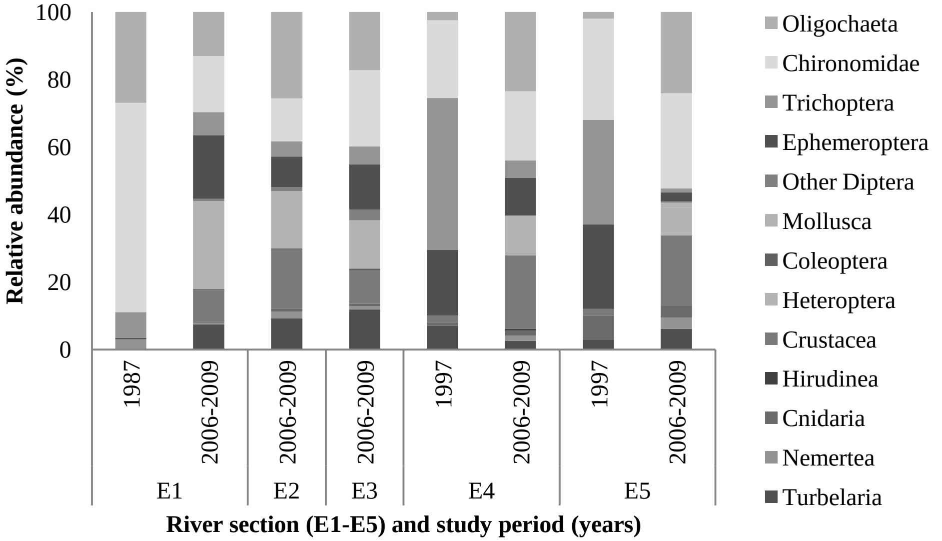

Fig. 2 Macroinvertebrate community structure (%) per section in the period 2006-2009 (this study), and comparison with previous studies carried out in section E1 (1987), E4 and E5 (1997).

Regarding macroinvertebrate diversity (Table 2), the highest values of species richness were found in summer (E4, 27 taxa) and the lowest number of species (12 taxa) occurred in spring (E5) and winter (E4). Diversity indices showed no significant differences among sections, but for Pielou's evenness index and Shannon-Wiener's diversity index, some significant differences among seasons were found (see Appendix 2).

Table 2. Macroinvertebrate community descriptive parameters for each sampling section. Richness (S), Shannon-Wiener's diversity index (H', as log_2) and Pielou's evenness index (J'). Includes value and category of IBMWP (Iberian Biological Monitoring Working Party).

	S	H'(log_2)	J'	IBMWP	
Spring				Value	Category
E1	19	2.39	0.56	84	Good
E2	17	1.54	0.38	55	Moderate
E3	21	2.47	0.56	85	Good
E4	14	1.87	0.49	40	Moderate
E5	12	1.98	0.55	29	Poor
Summer					
E1	19	3.00	0.71	82	Good
E2	20	3.37	0.78	82	Good
E3	20	2.91	0.68	87	Good
E4	27	3.70	0.78	112	Very good
E5	17	2.73	0.67	52	Moderate
Autumn					
E1	13	2.09	0.56	37	Moderate
E2	17	2.55	0.62	60	Moderate
E3	16	2.16	0.54	56	Moderate
E4	15	2.50	0.64	49	Moderate
E5	17	2.29	0.56	51	Moderate
Winter					
E1	14	2.75	0.72	43	Moderate
E2	21	2.55	0.58	83	Good
E3	18	3.22	0.77	70	Good
E4	12	2.78	0.78	42	Moderate
E5	15	2.85	0.73	51	Moderate

The mean values for IBMWP showed different seasonal ranges: in spring values ranged from 29 to 84, in summer fluctuated between 52 and 112, in autumn from 37 to 60 and during winter between 42 and 83. The highest value was found in summer at E4 indicating "very good" ecological status, whereas the lowest value was registered in spring at E5 indicating "poor" ecological status.

In terms of trophic structure, the dominant feeding guilds were scrappers (48.52%) followed by shredders (24.28%) and deposit feeders (20.48%). Seasonal changes due to the inter-annual variability showed a dominance of scrapers during spring (32.64%) and summer (42.89%), of shredders in winter (38.41%), and deposit feeders in autumn (47.04%). Appendix 1 **provides** a list of macroinvertebrate taxa found over the study period including feeding guilds and the sections where each taxon was found.

Macroinvertebrate MDS analysis (Fig. 3) showed two different communities, one corresponding to the section located next to the Flix dam (E5) and another that included sections located downstream (E1 E2 E3 E4), here after E1-4. Significant differences in community composition were found between E5 and E1-4 (ANOSIM r: 0.218, p=0.01), and also among seasons (ANOSIM r: 0.433, p=0.001) (spring $\neq$ summer $\neq$ autumn $\neq$ winter).

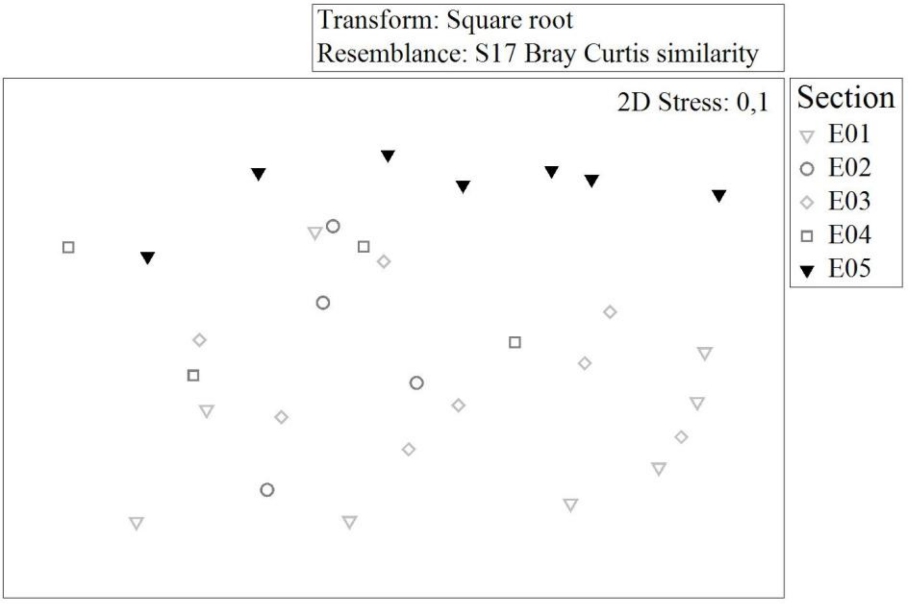

Fig. 3 Two dimensional MDS plots based on Bray-Curtis similarities of square-root transformed macroinvertebrate abundance data.

Similarity Percentages analysis SIMPER (Appendix 3) showed that the mean community similarity within E5 group was 42.57%, and the taxa that most contributed to the high similarity were Oligochaeta (21.75%), Orthocladiinae (15.62%), *Echinogammarus* (15.08%) and *Proasellus* (8.77%); a total of 11 taxa were necessary to accumulate 90% of similarity. The mean similarity within E1-4 group was 40.98% with a high contribution from Oligochaeta (15.84%), Orthocladiinae (14.27%), *Corbicula* (13.47%), *Echinogammarus* (9.46%) and *Baetis* (9.15%); 90% of similarity in this group was obtained with 14 taxa. Furthermore the mean dissimilarity between these two groups was 64.54% with Orthocladiinae, Oligochaeta, *Echinogammarus*, *Micronecta*, *Dugesia*, *Baetis* and *Corbicula* as the taxa with the highest contributions to dissimilarity.

BIOENV analysis showed that the combination of water temperature, substrate composition, dissolved oxygen, pH, conductivity and distance to the dam had the strongest influence on the structure of macroinvertebrate communities (ρ=0.378). These variables represented in a PCA (Fig. 4) explained in the two first axes 65.4% of the total variance. The first axis (37.5%) summarized variables displaying spatial and hydromorphological variation, where the distance to the dam was inversely proportional to the substrata size, which is related to the influence of the regulation system; these variables did not change along the study period. The second axis (27.9%) summarized the seasonal inter-annual variation, where the water temperature was opposed to dissolved oxygen levels.

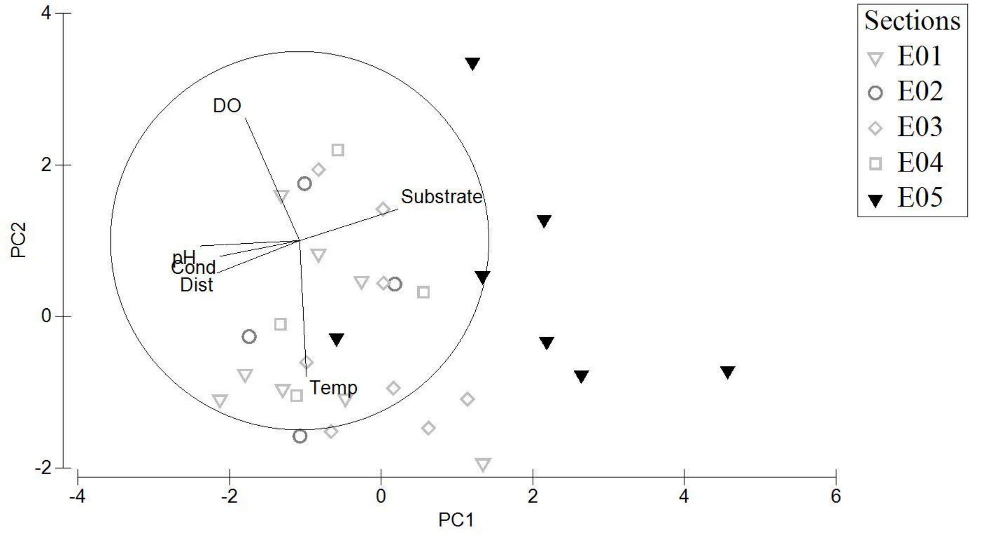

Fig. 4 PCA of the macroinvertebrate data showing the ordination of sampling sections as a function of the environmental variables

Diatom assemblages

A total of 124 diatom species were found during the study period. Only 67 had a relative abundance higher than 0.05% and therefore were used in the statistical analyses and are listed with their taxon authors and relative abundances in Appendix 4. Some species were present in all samples and abundant in many e.g. *Amphora pediculus*, *Cocconeis placentula* var. *lineata*, *Nitzschia dissipata* var. *dissipata*, *N. inconspicua* and *N. palea*. Others, such as *Navicula antonii* and *N. cryptotenella*, though rarely exceeding 10% relative abundance, were also present throughout. Clear changes occurred in the diatom community during the year: in summer, communities were mainly dominated by *Cocconeis placentula* var. lineata and *Nitzschia palea*, and in autumn by these two species as well as *Amphora pediculus*. In spring the diatom community was mainly dominated by *A. pediculus*, *Nitzschia dissipata* var. *dissipata* and *N. inconspicua*.

Concerning diatom diversity (Table 3), there was no clear pattern among river sections. However, there seemed to be a slight seasonal pattern with consistently higher species richness in autumn. The lowest number of species occurred in spring with the lowest value recorded (22) in E3. No significant differences in community diversity indices were observed among sections but some significant differences among seasons for IPS values were found (see Appendix 2).

Table 3. Diatom community descriptive parameters for each sampling section. Richness (S), Shannon-Wiener's diversity index (H', as log_2) and Pielou's evenness index (J'). Includes value and category of IPS (Specific Polluosensitivity Index)

	S	**H'(log_2)**	**J'**	**IPS**	
Spring				Value	Category
E1	27	3.45	0.74	14.40	Good
E2	29	3.32	0.68	13.90	Good
E3	22	2.62	0.58	14.87	Good
E4	23	3.33	0.74	13.70	Good
E5	26	2.88	0.62	12.40	Moderate
Summer					
E1	29	3.75	0.77	12.00	Moderate
E2	25	3.21	0.69	10.90	Moderate
E3	30	4.12	0.84	12.90	Moderate
E4	28	3.52	0.73	11.50	Moderate
E5	34	4.18	0.82	10.40	Moderate
Autumn					
E1	36	3.69	0.71	11.90	Moderate
E2	32	3.74	0.75	12.10	Good
E3	32	2.72	0.54	13.70	Good
E4	35	3.66	0.71	10.50	Moderate
E5	31	3.49	0.71	8.70	Poor

Diatom MDS analysis showed a defined pattern of distribution at seasonal scale (Fig. 5), and significant differences were found among seasons (ANOSIM r: 0.549, p=0.001) (spring ≠ summer ≠ autumn) but not among sections (ANOSIM r: -0.122, p=0.91). BIOENV analysis (ρ=0.482) showed a strong relation between the diatom community distribution and the combination of the following environmental variables: water temperature, pH, dissolved oxygen, soluble reactive phosphorus, SiO_4, chlorophyll and total dissolved nitrogen. These variables displayed in a PCA (Fig. 6) explained in the first two axes 61.2 % of total variance; the first axis (40.9%) summarized variables representing the seasonal variation, being water temperature opposed to dissolved oxygen and total dissolved nitrogen; and the second axis (20.3%) summarized the effects of dam regulation on water quality, displaying SiO_4 opposed to soluble reactive phosphorus, pH and chlorophyll.

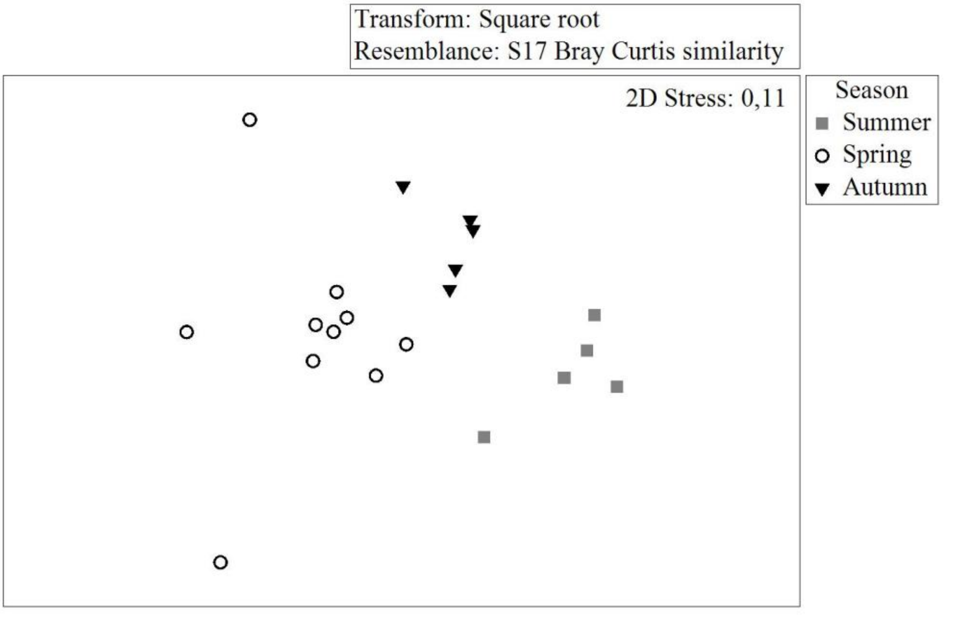

Fig. 5 Two dimensional MDS plots based on Bray-Curtis similarities of square-root transformed diatom abundance data

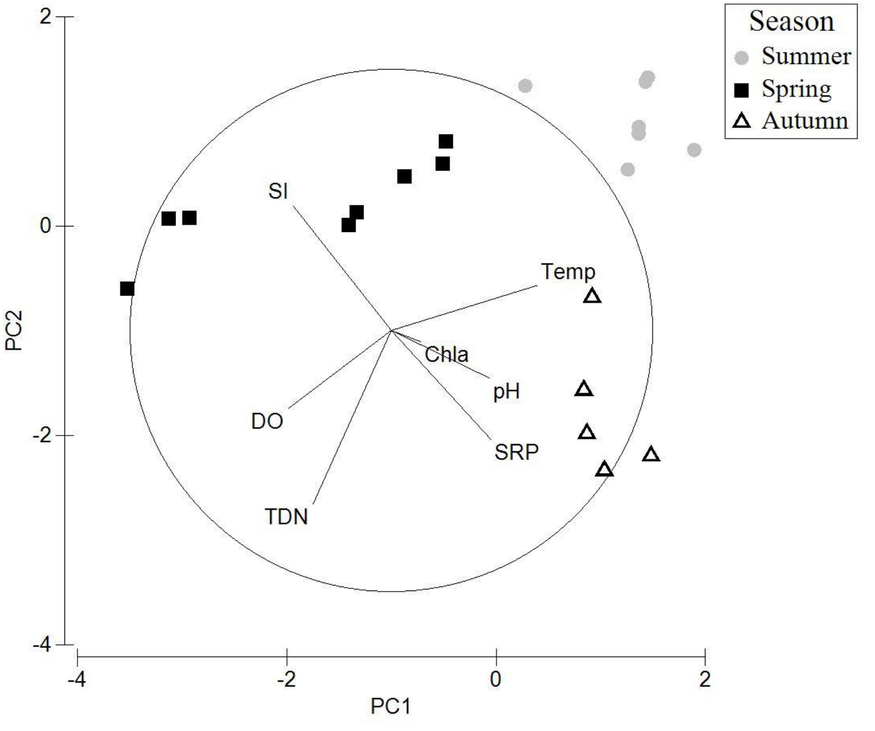

Fig. 6 PCA of the diatom data showing seasonal ordination as a function of the environmental variables.

Ecological status as measured by mean values of the IPS appeared to vary seasonally: in spring IPS values ranged from 12.40 to 14.40, in summer fluctuated between 10.40 and 12.90 and during autumn from 8.70 to 13.70.The highest values occurred in E3 and would indicate “good” (spring and autumn) and “moderate” (summer) ecological status. The lowest values were found in E5, indicating “poor” (autumn) and “moderate” (spring and summer) ecological status.

DISCUSSION

This work includes a complete characterization of benthic communities (macroinvertebrates and diatoms) in a large Mediterranean river using a methodology that covers all the morphological variability. Most of the existing studies have been performed in streams in part due to the difficulty in sampling benthic communities of large rivers, and often do not include the analysis of both macroinvertebrate and diatom communities, which have been widely used as biological indicators of ecological quality in rivers because they use to reflect different types of anthropogenic impacts.

The annual average water temperature (17.30^{o}C) found at E5 was lower than the value found at E1-4 (19.59 ^{o}C) and the seasonal water temperature variation was influenced by the presence of a nuclear power station located downstream E5 and by reservoirs located upstream. Their co-occurrence have opposite effects on the river water during summer and winter; this pattern has been previously documented by Prats *et al*. (2010) who noted that in summer the cooling effect of the reservoirs and the warming effect of the nuclear power station compensated each other; whereas in winter, the warming effect of both is added. Dissolved oxygen followed the same tendency with mean values of 87.75% and 94.67% for E5 and E1-4 respectively, and minimum summer daily values at E5 dropping down to 30%; this is due to the summer stratification of the reservoirs which release water from the hypolimnion with low oxygen and high nutrient content (Sabater *et al.*, 2008).

Benthic macroinvertebrate communities at the study area showed some significant spatial and temporal differences. The multivariate analysis defined two different communities as a function to distance to dams: the uppermost sampling section located close to the Flix dam (E5) was different from all the other sections located downstream (E1-4); this could be related with the direct influence of the Riba-roja and Flix dams at the first section of the stretch causing changes in substrate composition (coarser substrate due to erosion of fine materials) and water quality (low oxygen values and lower temperature), as well as higher chemical pollution (heavy metals, persistent organic compounds) due to the toxic waste accumulated in Flix reservoir (Cid *et al.*, 2010).

Results suggest that hydrogeomorphic variability was not a significant factor determining the structure of benthic communities in the lower Ebro River. Community structure was significantly different in the section closer to the regulation system and results of ecological status suggest that this was mostly due to the impact of dams rather than to the particular hydromorphic conditions of this section. This outcome is more in agreement with the conceptual framework of Serial Discontinuity (Ward & Stanford, 1983) proposed for regulated rivers.

Changes in abundance and diversity of macroinvertebrate fauna as consequence of flow regulation has been previously reported worldwide; for instance Poff and Zimmerman (2010) in a review of 165 papers published over the last four decades found that macroinvertebrates showed mixed responses to changes in flow magnitude, with abundance and diversity both increasing and decreasing in response to elevated flows and to reduced flows. Similar reports are found from studies in large Mediterranean rivers (e.g. Bournaud *et al.*, 1996; Chatzinikolaou *et al.*, 2006; Marchetti *et al.*, 2011; Muñoz & Prat, 1996) pointing the regulation system as one of the main anthropogenic alterations on macroinvertebrate communities. In fact, environmental flows are now considered as a key issue to achieve the good ecological status of superficial water bodies as required by the Water Framework Directive of the European Union (European Commission, 2012).

Although the level of nutrients has been reduced during the last decades (Ibáñez *et al.*, 2012), Chironomidae and Oligochaeta have a dominant status compared with groups as mayflies (Ephemeroptera) and caddisflies (Trichoptera). This pattern has been observed in streams and rivers with high levels of nutrients (Hawkes & Davies, 1971; Metcalfe, 1989; Whitehurst & Lindsey, 1990) and, in the lower Ebro this could be also enhanced by the presence of toxic pollution in the Flix reservoir. The macroinvertebrate community along all the study area was characterized in terms of abundance by taxa that somehow could be reflecting habitat degradation, such as Oligochaeta, Orthocladiinae, *Echinogammarus*, *Baetis*, *Corbicula*, *Dugesia* and *Caenis*. However, it is necessary a higher taxonomic identification conducted to species level in order to reach an adequate ecological interpretation; for instance, Oligochaeta has been traditionally categorized as tolerant taxa, but many intolerant Oligochaeta species can be eradicated as consequence of pollution; this evidence that only some Oligochaeta species are pollution tolerant (Lafont, 2011), in fact, biotic indices based in Oligochaeta assemblages have been used in approaches to the refinement of biomonitoring programs (Lafont *et al.*, 2010; Lafont *et al.*, 2012). The IBMWP, currently used by the WFD to assess the ecological status, has been developed based on traditional methodologies with low costs and considering a simple taxonomical identification; making it accessible and handy by all the member states. However, it is necessary to take in account the limitations and the loss of information as a result of not considering the identification to species level.

The macroinvertebrate composition obtained in this study is remarkably different when compared with the community found in 1987 at E1 as reported by Muñoz and Prat (1994) and at E4 and E5 as reported by Limnos (1997) (Fig.2). Although the methodology used was somewhat different, it is possible to recognize relevant changes in the community of the lower Ebro River; among the main changes it can be highlighted the diversification of the community, the decrease of Trichoptera (especially *Hydropsyche exocellata*), Chironomidae and other taxa indicating eutrophication; and the arrival of invasive species such as *Dreissena polymorpha* and *Corbicula fluminea.*

Historical changes in the density and production of *Ephoron virgo*, a filter feeding species that inhabits in fine gravel substrate, have been previously documented by Cid *et al*. (2008); it was registered as abundant in the 80's (Ibáñez *et al.*, 1991) and early 90's (Muñoz & Prat, 1994) but our results showed a decrease in abundance and presence which could be associated to the reduction in phytoplankton and the spread of the macrophyte pondweed *Potamogeton pectinatus* in the substrate occupied by this species. The first reports of the zebra mussel *Dreissena polymorpha* and the Asian clam *Corbicula fluminea* in the lower Ebro River date from 2001 and 1997, respectively; these species have quickly proliferated and in fact, during the study period specimens were found along all the sampling sections; these species of bivalves (filter feeders) may compete for food resources with native species such as Naiade, but their influence on the phytoplankton decline of the lower Ebro has been shown to be small in comparison to phosphorus decrease (Ibáñez *et al.*, 2012).

The distribution pattern of the diatom assemblage was clearly influenced by seasonal variation, and this temporal variability is related to the fluctuating along-year conditions of the lower Ebro River which involves variation in sunlight intensity, changes in water temperature and differences in nutrient concentrations, as well as changes in flow regulation. However, contrary to results obtained for macroinvertebrates, there were no significant differences among sections along the study area. This is consistent with the fact that macroinvertebrates and diatoms respond rather differently to anthropogenic stressors, being macroinvertebrates more sensitive to physical changes in river habitat, while diatoms are more sensitive to water quality alterations (Hering *et al.*, 2006; Pace *et al.*, 2012; Soininen & Könönen, 2004; Triest *et al.*, 2001).

The most abundant diatom species in the study area (*Amphora pediculus*, *Cocconeis placentula* var. *lineata*, *Nitzschia dissipata*, *N. inconspicua* and *N. palea*) are also common further upstream in the Ebro River (http://www.chebro.es/contenido.visualizar.do?idContenido=27971&idMenu=4101) as well as the fresher parts of its estuary (Rovira *et al.*, 2012a; Rovira *et al.*, 2012b), and more generally in many lowland rivers of Europe (e.g. Almeida & Feio, 2012; Urrea & Sabater, 2009; van Dam *et al.*, 2007). All the common species are widespread in α-,β- mesosaprobous waters (van Dam *et al.*, 1994).

Seasonal changes in diatom communities have also been found in rivers elsewhere (e.g. Goma *et al.*, 2005; Leira & Sabater, 2005; Martínez de Fabricius *et al.*, 2003; Sherwood *et al.*, 2000; Soininen & Eloranta, 2004). Changes are bound to occur in diatom communities during the year as a result of variation in light intensity, day-length, temperature and life cycles of grazers, but an extra factor in some Mediterranean rivers (e.g. Ebro, Po, Rhône) is the marked seasonal variation in flow as a result of snow-melt in spring and low summer precipitation (exacerbated by irrigation and industrial demand). Water flow variation could be a major factor controlling the seasonal changes observed in all sections analysed, consistent with the findings of Boix *et al*. (2010), Martínez de Fabricius *et al*. (2003) and Tang *et al*. (2013).

From the biomonitoring point of view it is interesting that all five sections had similar communities according to ANOSIM analysis and showed similar seasonal changes; this implies that all of them are representative of the whole stretch and therefore could be chosen for surveillance of the ecological status. However, the IPS scores were always lowest in section E5, the section closest to the regulation system, and at times indicated "poor" ecological status (in autumn) which is consistent with macroinvertebrate IBMWP scores, where again "poor" status was recorded only in E5 (although in spring). Otherwise, diatom (IPS) and macroinvertebrate (IBMWP) indices indicated "moderate" or "good" ecological status.

The fact that in many cases the ecological status was "good" according to diatoms and macroinvertebrates does not mean that the overall ecological status, including other biological indicators, can be considered to be "good" as well. For instance, the fish community of the lower Ebro River is strongly dominated by invasive species which are favored by dam regulation and river flow reduction (e.g. Bunn & Arthington, 2002; Gido *et al.*, 2013; Kiernan *et al.*, 2012; Lytle & Poff, 2004; Maceda-Veiga *et al.*, 2010; Olden *et al.*, 2006; Propst & Gido, 2004), and the ecological status of the lower Ebro River according to this indicator varies between "poor" and "bad" (Sostoa *et al.*, 2010). Furthermore, the absence of reference conditions in the lower Ebro regarding the biological communities before dam construction difficult the proper ecological quality assessment; however, even when reference conditions are well established, biological communities may also shift as consequence of factors as climate change.

No significant differences in community structure of diatoms and macroinvertebrates were found in sections E1 to E4, and the reason could be in part related to the methodology used for sampling, which did not cover all the internal variability of the river ecosystem since samples were collected in wadeable areas; thereby the littoral community is well represented, but we were not able to obtain information of the communities inhabiting the river channel due to the difficulty of sampling with high water flow and coarse substrate (trials with different types of dredges did not work). In addition, as Chironomidae and Oligochaeta were the dominant taxa in the macroinvertebrate community, higher taxonomic resolution for these groups may be necessary in future studies to be able to discriminate significant differences in community composition. We think that specific methods based on biological indicators capable of integrating responses to different impacts are needed in order to develop a more comprehensive assessment of the ecological status of large rivers subject to multi-stressor conditions.

CONCLUSIONS

The regulation system seems to be a main factor determining the structure of benthic communities in the lower Ebro River. The macroinvertebrate community along all the study area reflected in some way the habitat degradation still present at the lower Ebro River; and there were identified two different macroinvertebrate assemblages inhabiting the study area, as a function to distance to dams. While, the distribution pattern of the diatom assemblage was clearly influenced by the seasonal variation.

The scores of indices for ecological status assessment based on both, macroinvertebrates and diatoms, were lower in the section closest to reservoirs; even though, these two groups responded rather differently to anthropogenic stressors; macroinvertebrates were more sensitive to physical changes in river habitat, while diatoms were more sensitive to water quality alterations.

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SUPPLEMENTARY MATERIAL

APPENDIX 1. – List of macroinvertebrate taxa found over the study period in each of the sections (E1–E5). Feeding guilds included Absorber (A), Deposit feeder (D), Shredder (Sh), Scraper (Sc), Filter-feeder (F), Piercer (P), Predator (Pr) and Parasite (Ps).

Taxa		**Autumn**	**Winter**	**Spring**	**Feeding guild**
PHYLUM ANNELIDA					
Class Clitellata					
Dina	E3 E5				Pr
Helobdella	E1 E2 E4 E5	E1	E4	E1 E2 E3 E4 E5	P
Oligochaeta	E1 E2 E3 E4 E5	E1 E2 E3 E4 E5	E1 E2 E3 E4 E5	E1 E2 E3 E4 E5	D
PHYLUM ARTHROPODA					
Class Malacostraca					
Atyaephyra	E1 E3 E5		E2 E3 E5	E5	Sh
Echinogammarus	E1 E2 E3 E4 E5	E2 E3 E5	E1 E2 E3 E4 E5	E1 E2 E3 E4 E5	Sh
Proasellus	E1 E3 E4 E5	E5	E2 E3 E4 E5	E1 E2 E3 E4 E5	Sh
Class Insecta					
Baetis	E1 E2 E3 E4 E5	E1 E2 E3 E4	E1 E2 E3 E5	E1 E2 E3 E4	Sc
Caenis	E1 E2 E3 E4 E5	E1 E3 E4 E5	E1 E2 E3 E4 E5	E1 E2 E3 E4 E5	D
Ceraclea	E1 E3 E4		E2	E1 E3	Sh
Ceratopogoninae	E1 E5			E1	Pr
Ceriagrion	E3				Sh
Choroterpes	E1 E2 E3 E4			E1 E2 E3	D
Cloeon	E1 E2 E3 E5				D
Dryops	E1 E3 E4	E1 E2	E1 E2 E3	E1 E3	Sh
Ecdyonurus	E1			E1 E3	Sc
Ecnomus	E1 E2 E3 E4 E5	E2 E3 E4 E5	E3 E4 E5	E1 E3	F
Ephoron	E2 E3 E4			E1 E2 E3	F
Hexatomini				E1	Pr
Hydropsyche	E1 E2 E3 E4 E5	E3 E4 E5	E2 E3	E1 E3 E4	F
Hydroptila	E1 E2 E3 E4 E5	E2 E3 E5	E5	E1 E2 E3	P
Micronecta	E5	E4 E5	E5	E5	Sh
Mystacides	E1 E3				Sh
Orthotrichia	E1 E2 E3 E4	E2 E3			P
Platycnemis	E1 E3		E2 E3		Sh
Potamophilus	E3				Sh
Psychomyia	E1 E2 E3 E4	E4	E2 E4	E1 E3	Sc
Sisyra				E1 E3	P
Tipula				E1 E3	Sh
Orthocladiinae	E1 E2 E3 E4 E5	E1 E2 E3 E4 E5	E1 E2 E3 E4 E5	E1 E2 E3 E4 E5	Sc
Tanytarsini	E1 E2 E3 E4 E5	E1 E2 E4 E5	E2 E3 E5	E1 E2 E3	D
Tanypodinae	E1 E2 E3 E4 E5	E2 E4 E5	E1 E2	E1 E2 E3 E4 E5	Pr
Chironomini	E1 E2 E3 E4 E5	E3 E5	E1 E2 E3 E5	E1 E2 E4 E5	D
Dasyheleinae	E5				D
Hemerodromiinae	E1 E4			E01	Pr
Simuliinae	E1 E3	E1 E2 E3		E3	F

PHYLUM CNIDARIA					
Hydra	E3 E4 E5	E2 E3 E4 E5	E2 E3 E4	E2 E3 E4 E5	Pr
PHYLUM MOLLUSCA					
Class Bivalvia					
Corbicula	E1 E2 E3 E4 E5	E1 E2 E3 E4 E5	E1 E2 E3 E4 E5	E1 E2 E3 E4 E5	F
Dreissena	E1 E4 E5	E5	E5	E3 E4 E5	F
Class Gastropoda					
Ancylus	E5		E1		Sc
Ferrissia		E4 E5			Sc
Lymnaea	E1 E3 E4	E4	E2 E3	E3	Sc
Melanopsis	E1	E1		E1	Sc
Physella	E1 E3 E4 E5	E2 E3	E1 E2 E3 E4	E1 E2 E3	Sc
Theodoxus	E1 E3 E4	E1 E2	E1 E2 E3	E1 E3 E4	Sc
PHYLUM NEMERTEA					
Prostoma	E1 E2 E3 E5	E1 E2 E3 E4 E5	E1 E2	E1 E2 E3	Pr
PHYLUM PLATYHELMINTHES					
Dugesia	E1 E2 E3 E4 E5	E1 E2 E3 E4 E5	E1 E2 E3 E4 E5	E1 E2 E3 E4	Pr

APPENDIX 2. – One way-ANOVA test showing seasonal differences in diversity indices for macroinvertebrates and diatoms. Only results with signifficant values are shown.

Macroinvertebrates			*p*	Sig.
Pielou's evenness index	Spring	Summer	,000	***
	Spring	Winter	,001	***
	Summer	Spring	,000	***
	Summer	Autumn	,018	*
	Autumn	Summer	,018	*
	Autumn	Winter	,026	*
	Winter	Spring	,001	***
	Winter	Autumn	,026	*
Shannon-Wiener's diversity index	Spring	Summer	,000	***
	Spring	Winter	,006	**
	Summer	Spring	,000	***
	Summer	Autumn	,004	**
	Autumn	Summer	,004	**
	Winter	Spring	,006	**
Diatoms				
IPS	Spring	Summer	,044	*
	Spring	Autumn	,031	*
	Summer	Spring	,044	*
	Autumn	Spring	,031	*

***p* values:**
0,01 – 0,05 *
0,001 – 0,01 **
0 – 0,001 ***

APPENDIX 3. – Similarity Percentages analysis (SIMPER) of macroinvertebrate taxa showing mean community similarity within E5 and E1–4 groups; mean dissimilarity between these two groups and percentages of taxa contribution.

Group E1–4
Average similarity: 40.98

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Oligochaeta	10,65	6,49	1,39	15,84	15,84
Orthocladiinae	14,94	5,85	1,42	14,27	30,11
Corbicula	9,65	5,52	1,49	13,47	43,58
Echinogammarus	11,92	3,88	1,06	9,46	53,04
Baetis	10,73	3,75	1,33	9,15	62,19
Dugesia	7,86	3,1	1,13	7,57	69,76
Caenis	4,86	2,46	1,51	6	75,76
Theodoxus	5,7	1,27	0,76	3,09	78,85
Hydroptila	4,72	1,1	0,75	2,68	81,53
Hydropsyche	5,58	1,05	0,69	2,55	84,08
Physella	2,71	0,8	0,86	1,95	86,03
Tanytarsini	3,26	0,7	0,71	1,71	87,75
Prostoma	1,99	0,67	0,55	1,64	89,39
Tanypodinae	1,49	0,55	0,67	1,35	90,74

Group E5
Average similarity: 42.57

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Oligochaeta	18,34	9,26	2,06	21,75	21,75
Orthocladiinae	20,36	6,65	1,81	15,62	37,37
Echinogammarus	15,87	6,42	2,14	15,08	52,45
Proasellus	7,38	3,73	1,82	8,77	61,22
Dugesia	9,4	2,81	1,1	6,6	67,82
Micronecta	10,52	2,52	0,8	5,93	73,75
Tanytarsini	6,73	1,83	0,72	4,3	78,05
Caenis	4,72	1,68	0,92	3,96	82
Hydroptila	3,54	1,24	1,33	2,92	84,92
Chironomini	4,02	1,22	1,13	2,87	87,79
Tanypodinae	3,96	1,14	1,25	2,67	90,46

Groups E1–4 & E5
Average dissimilarity = 64.54

Species	Group E1–4 Av.Abund	Group E5 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Orthocladiinae	14,94	20,36	7,29	1,18	11,29	11,29
Oligochaeta	10,65	18,34	5,63	1,38	8,73	20,02
Echinogammarus	11,92	15,87	5,59	1,33	8,65	28,67
Micronecta	0,07	10,52	4,18	1,03	6,47	35,14
Dugesia	7,86	9,4	3,85	1,2	5,97	41,11
Baetis	10,73	0,55	3,82	1,12	5,92	47,03
Corbicula	9,65	2,1	3,33	1,05	5,16	52,2
Hydra	1,4	7,69	2,84	0,71	4,41	56,6
Proasellus	1,09	7,38	2,82	1,41	4,36	60,97
Tanytarsini	3,26	6,73	2,79	1,26	4,33	65,29
Caenis	4,86	4,72	2,11	1,21	3,28	68,57
Theodoxus	5,7	0	2,04	0,8	3,17	71,73
Hydropsyche	5,58	1,38	1,92	0,85	2,97	74,71
Prostoma	1,99	3,33	1,92	0,69	2,97	77,68
Hydroptila	4,72	3,54	1,81	1,37	2,8	80,48
Chironomini	1,37	4,02	1,54	1,09	2,38	82,86
Tanypodinae	1,49	3,96	1,46	1	2,26	85,12
Physella	2,71	1,13	1,07	1	1,65	86,77
Dreissena	0,26	2,59	0,99	1,25	1,53	88,3
Simuliinae	2,76	0	0,95	0,51	1,47	89,77
Ecnomus	1,45	1,38	0,88	0,95	1,36	91,13

APPENDIX 4. – List of diatom taxa found and their relative abundances over the study period in each of the sections (E1–E5).

Taxa	Spring					Summer					Autumn				
	E1	**E2**	**E3**	**E4**	**E5**	**E1**	**E2**	**E3**	**E4**	**E5**	**E1**	**E2**	**E3**	**E4**	**E5**
Achnanthes conspicua A. Mayer	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Achnanthes lanceolata ssp. *frequentissima* var. *rostrata* (Oestrup) Hustedt	0.0	0.0	0.0	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Achnanthidium minutissimum (Kützing) Czarnecki	5.6	0.7	26.8	2.6	0.8	0.3	0.2	0.7	1.2	6.6	0.0	0.0	0.0	0.0	0.0
Adlafia minuscula (Grunow) Lange-Bertalot	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0
Amphora inariensis Krammer	0.0	0.0	0.0	0.0	0.0	1.3	0.0	1.4	1.9	2.0	0.0	0.0	0.0	0.0	0.0
Amphora libyca Ehrenberg	0.7	0.0	0.2	0.0	0.0	3.1	0.4	4.8	1.6	1.0	0.0	0.0	0.0	0.7	0.0
Amphora ovalis (Kützing) Kützing	1.1	0.2	0.5	0.0	0.8	3.4	0.4	1.4	0.5	0.5	0.0	1.6	0.0	0.5	0.5
Amphora pediculus (Kützing) Grunow	24.6	18.8	19.3	20.9	43.8	4.9	0.7	10.0	1.6	7.1	19.7	19.8	24.8	20.0	31.6
Bacillaria paxillifer var. *paxillifer* (O.F. Müller) Hendey	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.4	0.2	0.2	0.2	0.2
Cocconeis neothumensis Krammer	0.0	0.0	0.0	0.0	0.2	0.0	0.0	6.7	1.4	0.5	0.0	0.0	0.0	0.0	0.0
Cocconeis pediculus Ehrenberg	1.3	0.0	0.5	0.0	0.0	0.3	0.2	0.5	1.9	2.8	0.9	0.7	0.2	0.5	0.2
Cocconeis placentula var. *lineata* (Ehrenberg) Van Heurck	4.3	1.2	0.9	0.4	0.5	20.4	13.8	20.1	17.4	5.8	21.2	23.0	46.2	8.0	8.4
Cyclotella meneghiniana Kützing	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.2	0.0	0.0	0.5	1.5	0.2	0.7
Diadesmis confervacea Kützing	0.0	0.5	0.1	3.3	0.1	0.3	0.2	0.5	0.0	2.3	0.4	0.7	0.5	5.5	17.6
Diatoma vulgaris Bory	0.8	2.2	0.7	0.7	0.2	0.0	0.2	0.0	0.0	1.5	0.0	0.0	0.0	0.0	0.7
Eolimna minima (Grunow) Lange-Bertalot	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0	0.0	0.2
Eolimna subminuscula (Manguin) Moser, Lange-Bertalot & Metzeltin	0.2	0.0	0.3	0.0	0.3	2.6	4.7	1.4	11.5	2.8	3.1	3.0	1.0	0.9	0.9
Fallacia lenzii (Hustedt) Lange-Bertalot	0.3	0.5	0.1	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fistulifera pelliculosa (Brébisson) Lange-Bertalot	0.4	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fistulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot	0.3	1.5	0.1	0.0	0.0	0.0	2.4	0.0	0.2	0.0	0.9	0.0	0.0	0.0	0.0
Fragilaria capucina var. *capucina* Desmazières	0.1	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.5	0.7	0.7	0.2	0.2	0.7
Gomphonema angustatum (Kützing) Rabenhorst	0.6	0.5	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	2.4	0.5	0.5	1.1	2.3
Gomphonema minutum f. *minutum* (C. Agardh) C. Agardh	0.6	0.0	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gomphonema olivaceum var. *olivaceum* (Hornemann) Brebisson	0.2	3.2	0.4	5.5	0.5	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gomphonema parvulum var. *parvulum* (Kützing) Kützing	0.6	0.0	0.0	3.5	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0
Gomphonema sp.	0.3	2.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.5	2.3
Gomphonema truncatum Ehrenberg	0.0	0.0	0.0	0.0	0.0	1.0	0.2	0.5	0.5	0.5	0.0	0.0	0.0	0.0	0.0
Gyrosigma nodiferum (Grunow) Reimer	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.7	0.2	0.2	0.2

Taxa	Spring					Summer					Autumn				
	E1	E2	E3	E4	E5	E1	E2	E3	E4	E5	E1	E2	E3	E4	E5
Karayevia clevei (Grunow) Round & Bukhtiyarova	0.3	0.0	0.0	0.0	0.4	0.0	0.0	5.3	0.0	0.3	0.2	0.0	1.2	0.0	0.0
Melosira varians C. Agardh	1.3	2.5	1.1	3.1	0.8	0.0	0.0	0.0	0.0	12.4	0.4	0.5	0.7	1.4	8.8
Navicula antonii Lange-Bertalot	1.7	4.5	4.7	3.1	2.4	6.2	3.6	8.1	6.6	2.8	0.7	8.4	2.2	5.5	0.5
Navicula capitatoradiata Germain	0.5	0.2	0.4	0.2	0.2	0.0	0.2	0.2	0.5	0.0	0.7	0.2	1.0	0.5	0.2
Navicula cryptotenella Lange-Bertalot	6.2	2.7	4.4	2.6	1.7	11.4	5.1	4.8	5.2	1.5	4.6	4.3	1.5	2.7	1.4
Navicula cryptotenelloides Lange-Bertalot	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Navicula erifuga Lange-Bertalot	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.2	0.0	0.0
Navicula lanceolata (C. Agardh) Ehrenberg	0.2	0.5	0.1	0.7	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Navicula recens (Lange-Bertalot) Lange-Bertalot	0.2	0.2	0.3	0.4	0.1	0.0	0.0	0.5	0.0	0.0	0.9	1.4	0.5	0.0	0.7
Navicula reichardtiana var. *reichardtiana* Lange-Bertalot	0.2	1.7	0.2	2.6	0.5	2.1	0.0	0.0	0.0	0.8	1.1	0.2	0.2	0.0	0.2
Navicula sp.	0.6	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Navicula subhamulata Grunow	0.1	0.0	0.1	0.0	0.0	0.3	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Navicula tripunctata (O.F.Müller) Bory	1.8	0.7	0.4	0.0	0.3	0.0	0.0	0.0	0.5	0.3	0.4	0.2	0.5	0.7	2.1
Navicula upsaliensis (Grunow) Peragallo	0.1	0.0	0.1	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Navicula veneta Kützing	0.4	0.0	0.3	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.7	0.2	0.2	1.1	0.5
Navicula viridula (Kützing) Ehrenberg	0.0	0.0	0.0	0.0	0.0	0.8	1.1	3.6	1.2	0.0	0.0	0.0	0.0	0.0	0.0
Navicula viridula var.*rostellata* (Kützing) Cleve	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.2	0.5	0.0
Nitzschia amphibia f. *amphibia* Grunow	0.1	0.2	0.0	0.2	0.7	4.1	3.3	2.4	1.4	15.9	0.2	0.0	0.0	0.5	0.0
Nitzschia dissipata var. *dissipata* (Kützing) Grunow	15.7	26.0	15.3	28.4	12.1	2.6	2.4	3.1	5.9	0.5	1.8	9.8	3.6	9.8	0.5
Nitzschia filiformis var. *filiformis* (W.M.Smith) Van Heurck	0.0	0.0	0.0	0.0	0.0	1.6	2.9	1.9	1.4	0.3	0.0	0.2	0.0	0.0	0.0
Nitzschia fonticola (Grunow) Grunow	3.5	3.2	4.1	3.1	0.9	2.1	21.8	2.4	2.8	7.3	0.4	2.5	0.7	2.5	0.7
Nitzschia frustulum var. *frustulum* (Kützing) Grunow	1.1	0.0	1.0	0.2	0.5	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.2	0.5	0.7
Nitzschia inconspicua Grunow	9.6	22.8	10.3	13.2	10.1	1.8	3.1	2.4	1.9	7.1	7.7	5.9	1.7	4.5	5.3
Nitzschia microcephala Grunow	0.2	0.5	2.0	0.0	2.0	0.8	0.4	0.2	1.6	3.0	0.2	2.7	0.2	2.3	1.4
Nitzschia palea (Kützing) W. Smith	1.1	0.5	1.7	1.8	0.2	22.2	30.1	8.1	29.6	0.5	18.6	2.7	6.8	25.2	0.7
Nitzschia sp.	0.4	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	0.1	0.2	0.1	0.0	0.3	0.3	0.0	3.1	0.2	0.3	0.9	0.0	0.7	0.2	0.0
Pleurosira laevis f. *laevis* (Ehrenberg) Compère	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.2	0.2	0.2	0.2	0.0
Pseudostaurosira brevistriata (Grunow) D.M Williams & Round	0.0	0.0	0.0	0.0	0.0	0.8	0.0	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Taxa	Spring					Summer					Autumn				
	E1	**E2**	**E3**	**E4**	**E5**	**E1**	**E2**	**E3**	**E4**	**E5**	**E1**	**E2**	**E3**	**E4**	**E5**
Reimeria uniseriata Sala, Guerrero & Ferrario	0.0	0.0	0.1	0.0	0.6	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot	11.1	1.0	1.6	2.6	0.6	1.3	0.0	0.0	0.9	1.8	1.5	1.6	0.5	0.7	2.1
Sellaphora seminulum (Grunow) D.G. Mann	0.1	0.0	0.0	0.0	6.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Seminavis ventricosa (Gregory) Garcia-Baptista	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.9	0.0	1.1	0.0
Staurosira construens Ehrenberg	0.2	0.0	0.3	0.0	4.0	0.8	0.0	0.0	0.0	0.5	1.1	0.2	0.5	0.5	4.4
Staurosira construens f. *subsalina* (Hustedt) Bukhtiyarova	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0
Staurosira elliptica (Schumann) D.M. Williams & Round	0.0	0.0	0.0	0.0	0.1	2.8	1.8	2.9	0.2	8.3	0.0	0.0	0.0	0.0	0.0
Tabularia fasciculata (C. Agardh) D.M. Williams & Round	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.2	0.0
Thalassiosira pseudonana Hasle & Heimdal	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	3.4	0.7	0.9	3.2
Ulnaria ulna (Nitzsch) Compère	0.1	0.0	0.0	0.0	0.0	0.3	0.2	0.0	0.2	0.3	0.0	0.0	0.0	0.0	0.0

Chapter 2

Benthic diatom communities of a large Mediterranean river under the influence of a thermal effluent

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***Limnetica* (In review)**

Benthic diatom communities of a large Mediterranean river under the influence of a thermal effluent

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ABSTRACT

The influence of a thermal discharge caused by the cooling system of a Nuclear Power Station on benthic diatom communities was assessed at the lower Ebro River (in Spain), and the information generated could be useful to assess the impacts of global warming on large Mediterranean rivers.

Surveys conducted at sites before and after the effluent and collected from natural and artificial substrate were analyzed and, Non-metrical Multidimensional Scaling (NMDS), Similarity Percentage Analysis (SIMPER) and 1-way Analysis of Similarities (ANOSIM) were performed to assess changes in community structure. The relationship between diatom 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).

Diatoms showed sensitivity to thermal changes, even though when these did not exceed 3 ^{o}C. The factors that seemed to influence benthic assemblages the most were seasonal variation and the thermal increase caused by the Nuclear Power Station.

INTRODUCTION

Diatoms are unicellular algae with a wide spectrum of responses to seasonal and environmental variation, and with optimum ranges of temperature to grow (Hawkes, 1969; Patrick, 1969; 1971). Each species has different tolerances and preferences, and some have therefore been used as indicators of environmental changes and conditions (Chessman *et al*., 1999; McCormick & Cairns Jr, 1994; Whitton & Kelly, 1995).

Temperature has a significant role in all biochemical and physiological functions of organisms and influences the morphology, physiology, behavior, growth, reproduction, and distribution of species (De Nicola, 1996; Kishi *et al*., 2005), and it has been noted as a main factor influencing primary production (e.g. Dallas, 2008). In fact, the rate of photosynthesis depends directly on temperature because it is an enzyme controlled process (Raven & Geider, 1988). It has been reported that warming generally increases the primary production (Kishi *et al*., 2005).

The importance of temperature in rivers has been widely recognized (Caissie, 2006; Dallas, 2008; Ward, 1985), and the effects of its alteration on aquatic species cover a wide spectrum of direct and indirect effects that range from minor importance to lethal effects (Verones *et al*., 2010). Changes in community structure as response to thermal disturbances have been detected even with a temperature alteration of few degrees centigrade (Kaushal *et al*., 2010) and depend on the preference and tolerance of species to different temperatures as well as on the level of heating.

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 been shown to alter biological and ecological components of aquatic ecosystems (Caissie, 2006; de Vries *et al*., 2008; Langford, 1990), but the effects are variable, and depend on the levels and quantity of heated discharge and on the biological features of the environment (Lardicci *et al*., 1999; Teixeira *et al*., 2010). Depending on the design and the operating units of the power plants, water temperature in effluent sites can increase by as much as 8 ^{o}C (Laws, 1993). However, in Europe, legislation requires that the temperature downstream of the effluent should not increase by more than 3^{o}C (European Union, 2006).

Many authors have studied the ecological effects of temperature in aquatic environments (e.g. de Vries *et al*., 2008; Hawkes, 1969; Verones *et al*., 2010), and several such studies have been based on diatoms (De Nicola, 1996; Patrick, 1969; Potapova & Charles, 2002; Raven & Geider, 1988). Furthermore, diatoms have been also used as biological proxy to assess the effects of climate change (Capítulo *et al*., 2010; McCormick & Cairns Jr, 1994; Perkins *et al*., 2010; Stevenson & Sabater, 2010; Wrona *et al*., 2006). The impacts of thermal effluents on benthic diatom communities have been studied mostly in estuarine and coastal regions (Hein & Koppen, 1979; Hillebrand *et al*., 2010; Lardicci *et al*., 1999; Snoeijs & Prentice, 1989; Teixeira *et al*., 2010), and to a lesser extent in lakes, rivers and streams (Boylen & Brock, 1973; Hickman & Klarer, 1975; Lamberti & Resh, 1985; Squires *et al*., 1979; Vinson & Rushforth, 1989). However, literature dealing with the effects of thermal pollution on benthic diatom communities of Mediterranean rivers is absent, even though this type of alteration is frequent in the watersheds of the Mediterranean basin.

This study aimed to assess changes in the community structure of benthic diatoms caused by the thermal pollution due to the cooling system of a nuclear power station (Ascó Nuclear Power Station). 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. For this purpose, 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.

MATERIALS AND METHODS

Study area

The Ebro River, located in the NE of the Iberian Peninsula (Fig. 1a) has a length of 928 km; its basin has a surface of 85 534 km^2 being one of the most important tributaries to the Mediterranean Sea, and over 180 dams regulate the river flow. 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. The last downstream dam is located at Flix, a small reservoir with a capacity of 11.4 hm^3.

The Ascó nuclear power station is located at 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 Delta (Fig. 1b). It was built in 1984 and has two reactors with a gross 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 granted a concession of 72.3 m^3/s of the Ebro's flow for its cooling system, and a weir has been 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 (Prats *et al*., 2010).

The study area has a total length of 2 km that comprise 1 km before and after the Nuclear Power Station, a mean width of 140 m and the substrate is dominated by gravel.

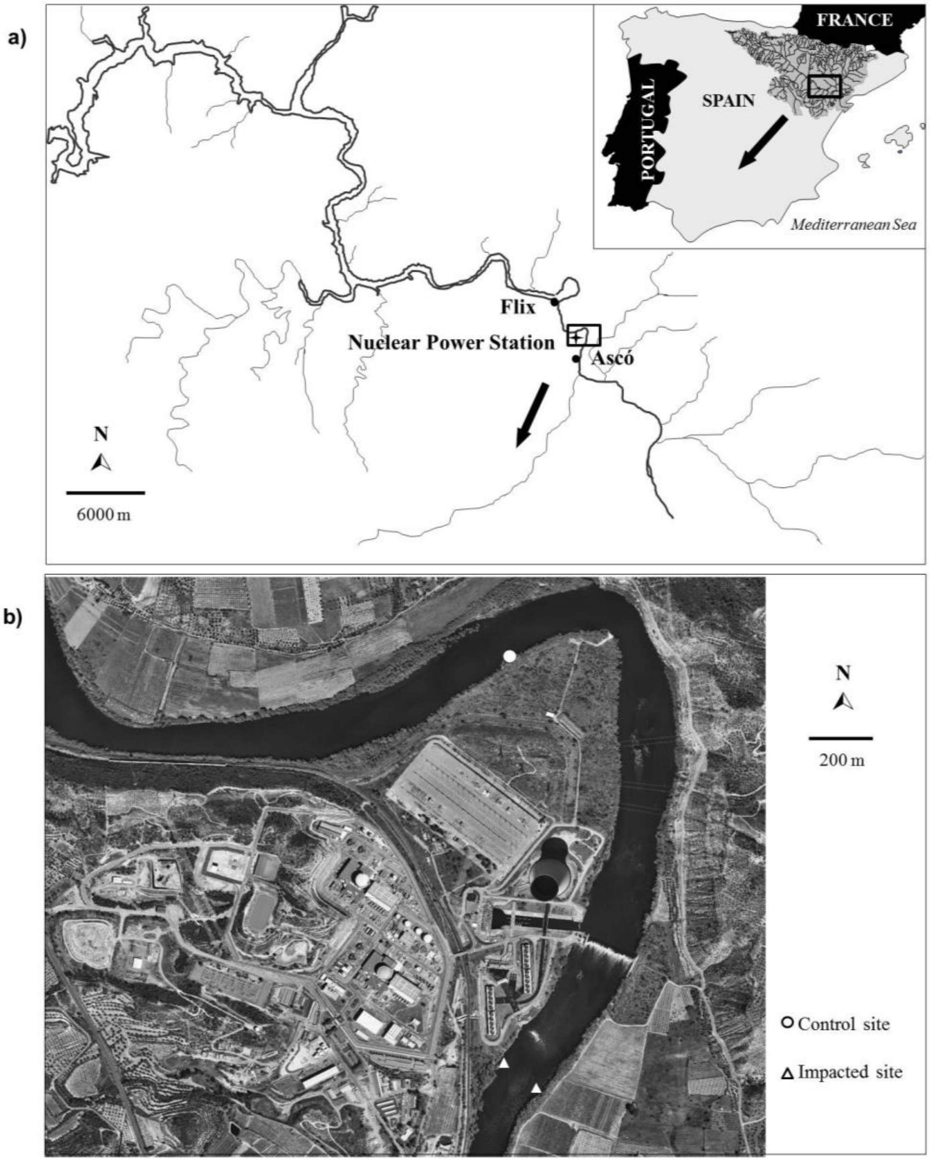

Figure 1. a) Map of the lower Ebro River showing the study area, b) location of sampling sites.

Diatom sampling and preparation

In order to compare benthic community features of a site unimpacted by the heated effluent with those under its influence, 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).

Three sampling campaigns were conducted in August, October and December of 2013. In every occasion, three replicates were collected at each site from both natural substrata (pebbles) and artificial substrata (fired clay bricks placed with a colonization period of 6 weeks). During the summer campaign the artificial substrates placed on site I1 were not recovered due to vandalism.

For every sampling site and occasion, physicochemical data were recorded. 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 (1977; 1983); and the total chlorophyll concentration was calculate using the colorimetric method (Jeffrey & Humphrey, 1975). In each sampling site, water temperature (°C) was monitored at intervals of 30 minutes during all the study period with a TCtemp1000 data logger Madgetech.

Benthic diatom samples were collected according to the recommendations of Kelly *et al*. (1998). The suspension was fixed in 4% formaldehyde solution. At the laboratory, benthic samples were oxidized with H_2O_2 30% v/v for several hours in order to remove the organic matter. HCl^- 37% v/v was added to evaporate the carbonates from the samples, as described in Renberg (1990).

Clean valves were permanently mounted with Naphrax© (refractive index 1.74). The permanent slides were examined using a LEICA DMI 3000 B light microscope equipped with differential interference contrast (DIC) with a 100 times oil immersion objective (n.a = 1.40).

Identification of diatoms was done to species level mainly following Krammer and Lange-Bertalot (1986–1991) but other taxonomic and floristic works were also used when needed. A minimum of 400 valves were counted each time.

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 variability at each site was represented by the standard deviation values (TempSD).

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

Diatom 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, relationship between diatom assemblages and environmental variables was assessed with a multivariate distanced-based linear regression model (DISTLM) (McArdle & Anderson, 2001) and a set of explanatory variables was identified. The model was visualized through a distance-based redundancy analysis (dbRDA) performed using PRIMER V6 software (Clarke & Gorley, 2006) with the add-on package PERMANOVA+ (Anderson *et al*., 2008).

RESULTS

Environmental characteristics

The average values for physicochemical parameters measured at each sampling site are shown in Table 1. Water temperature showed constantly higher values at impacted sites as consequence of the water heating produced by the cooling system of the Nuclear Power Station (Fig. 2), and was significantly different 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 Tº 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 measured environmental variables (dissolved oxygen, pH, conductivity, soluble reactive phosphorus, total phosphorus, total dissolved nitrogen, total nitrogen and depth) only showed minor variation and did not present significant differences between sites (ANOVA p>0.05). Dissolved oxygen at control site showed highest values than impacted sites in winter (10.23 mg/l) and in summer (8.46 mg/l) but in autumn I2 showed the highest value (7.71 mg/l); pH showed variation from 7.8 to 8.1; conductivity varied from 0.84 mS/cm in summer (C) to 1.31 mS/cm in winter (I2); soluble reactive phosphorus showed a minimum of 29.6 μg/l (C, winter) and a maximum of 53μg/l (I1, summer); total phosphorus ranged from 111.5 μg/l (C, winter) to 598 μg/l (I2, summer); total dissolved nitrogen varied from 1319.4 μg/l (I1, autumn) to 1712.2 μg/l (I1, winter); total nitrogen showed a minimum of 1999.9 μg/l (I1, autumn) and a maximum of 3120.4 μg/l (I1, winter); and the mean depth values recorded were 0.74 m (C), 0.74 m (I1) and 0.80 m (I2).

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)
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
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
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
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
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
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
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
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
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

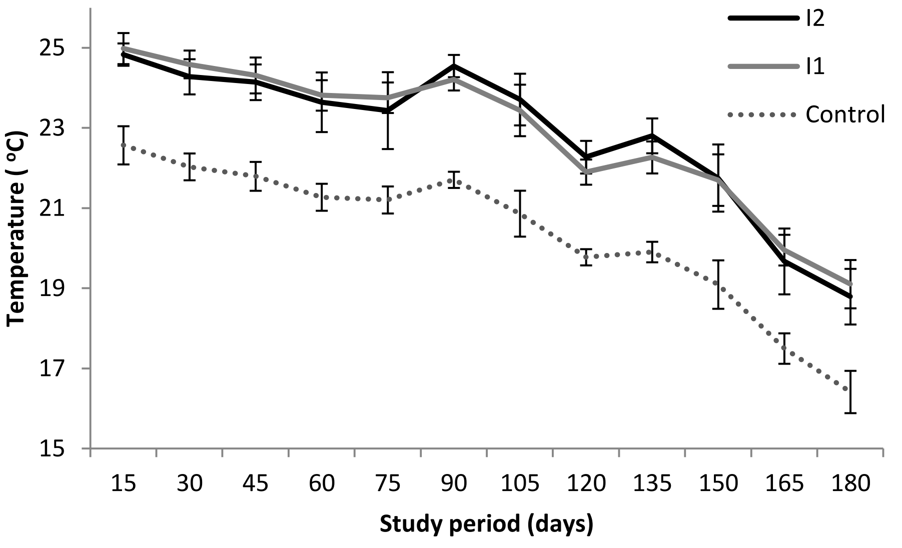

Figure 2. Water temperature recorded over the study period at control (C) and impacted (I1, I2) sites.

Diatom assemblages

During the study period a total of 85 species in natural substrate and 78 species in artificial substrate were found and are listed with their taxon authors and relative abundances in Appendix 1. Seasonal changes were observed in the diatom community along the study period. In natural substrate assemblages, *Amphora pediculus* and *Nitzschia inconspicua* were the dominant species, sharing this dominance with *Navicula capitatoradiata* in summer, with *Amphora copulata* in autumn, and with *Reimeria uniseriata* in winter. Artificial substrate assemblages were dominated in summer by *Nitzschia inconspicua, N. palea* and *Cocconeis placentula* var. *lineata*; in autumn by *Amphora pediculus, Cocconeis placentula* var. *euglypta* and *Nitzschia inconspicua;* and in winter by *Amphora pediculus, Cocconeis placentula* var. *lineata* and *C. placentula* var. *trilineata.*

Concerning diatom diversity (Appendix 2), there were no significant differences (ANOVA $p>0.05$) between control and impacted sites. However, when mean annual values were analyzed, slightly higher values of species richness and diversity indices were found at impacted sites (Appendix 3).

The NMDS ordination (Fig. 3) displays the spatial distribution of the control (C) and impacted sites (I1, I2); the stress obtained was 0.18 and 0.17 for natural and artificial substrates respectively. For both types of substrate, the assemblage composition was analyzed with ANOSIM and showed significant differences between C and I1, and between C and I2; but not between I1 and I2 (Table 2).

Table 2. Values of R statistic and significance level of differences between C, I1 and I2 groups, obtained by ANOSIM test for diatom communities of natural and artificial substrate.

Groups	R statistic	Significance	
Natural Substrate			
C, I1	0.196	0.010	**
C, I2	0.299	0.005	**
I1, I2	0.083	0.11	
Artificial Substrate			
C, I1	0.442	0.0003	***
C, I2	0.323	0.017	*
I1, I2	0.213	0.05	

Significance: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

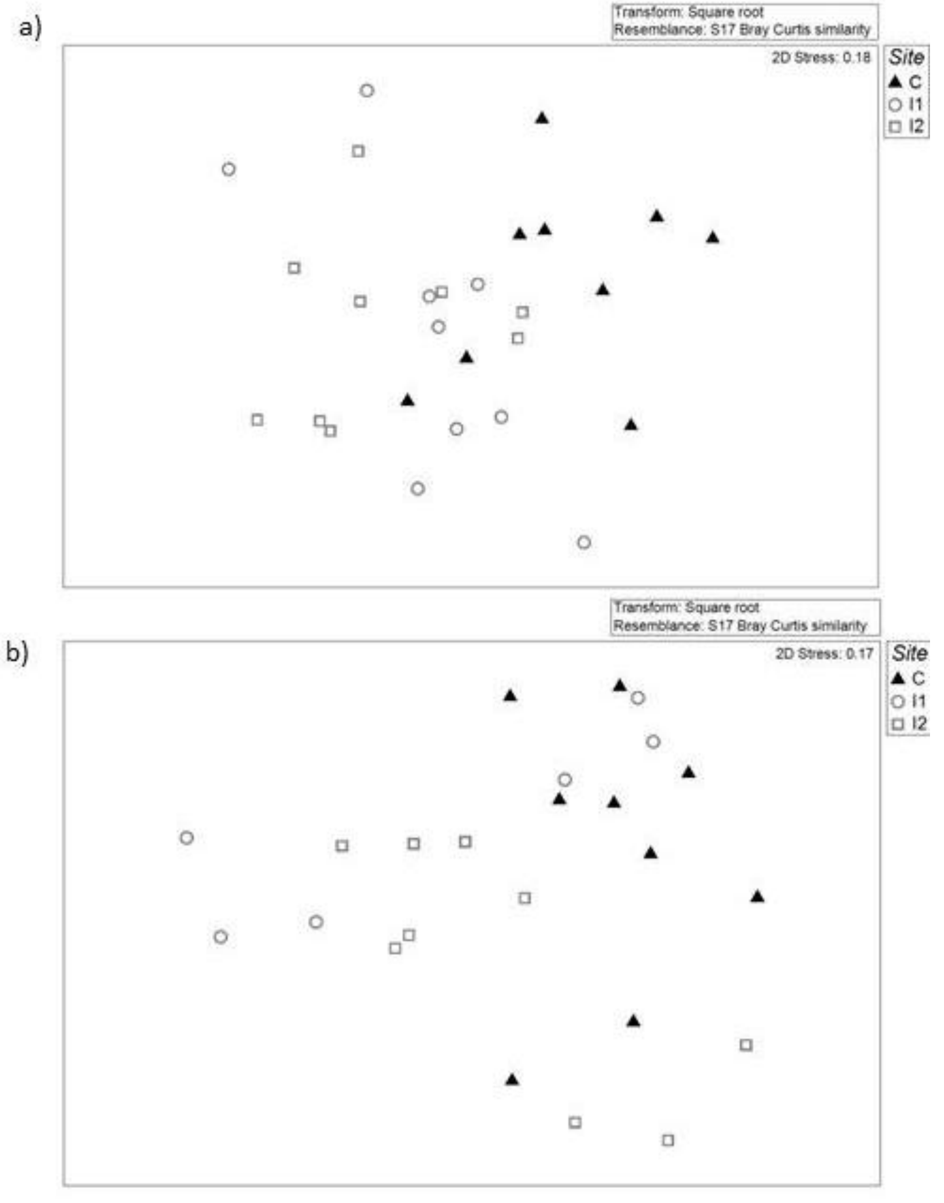

Figure 3. Two dimensional NMDS plots based on Bray-Curtis similarities of square-root transformed diatom abundance data. a) Natural substrate ordination. b) Artificial substrate ordination.

The Simper analysis (Appendix 3) for natural substrate assemblages showed that the mean dissimilarity between control and impacted sites was 42.22 % and *Amphora pediculus*, *Nitzschia inconspicua* and *Navicula capitatoradiata* were the species with highest percentage of contribution to dissimilarity between groups. While for artificial substrate, the mean dissimilarly was 39.97 % and the species with the highest contribution were *Amphora pediculus*, *Nitzschia inconspicua* and *Rhoicosphenia abbreviata.*

The dbRDA analysis performed for natural substrate (Fig. 4), revealed that the set of variables selected by the DISTLM (Tº, total nitrogen, Tº difference, chlorophyll and Tº variability) explained 57.27 % of fitted variation and 26.81 % 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 (dissolved oxygen, Tº difference, total phosphorus, pH and chlorophyll) explained 67.24 % of fitted variation and 39.55 % of total variation in the first two axes. Water velocity was not selected by the DISTLM as part of the explanatory variables set.

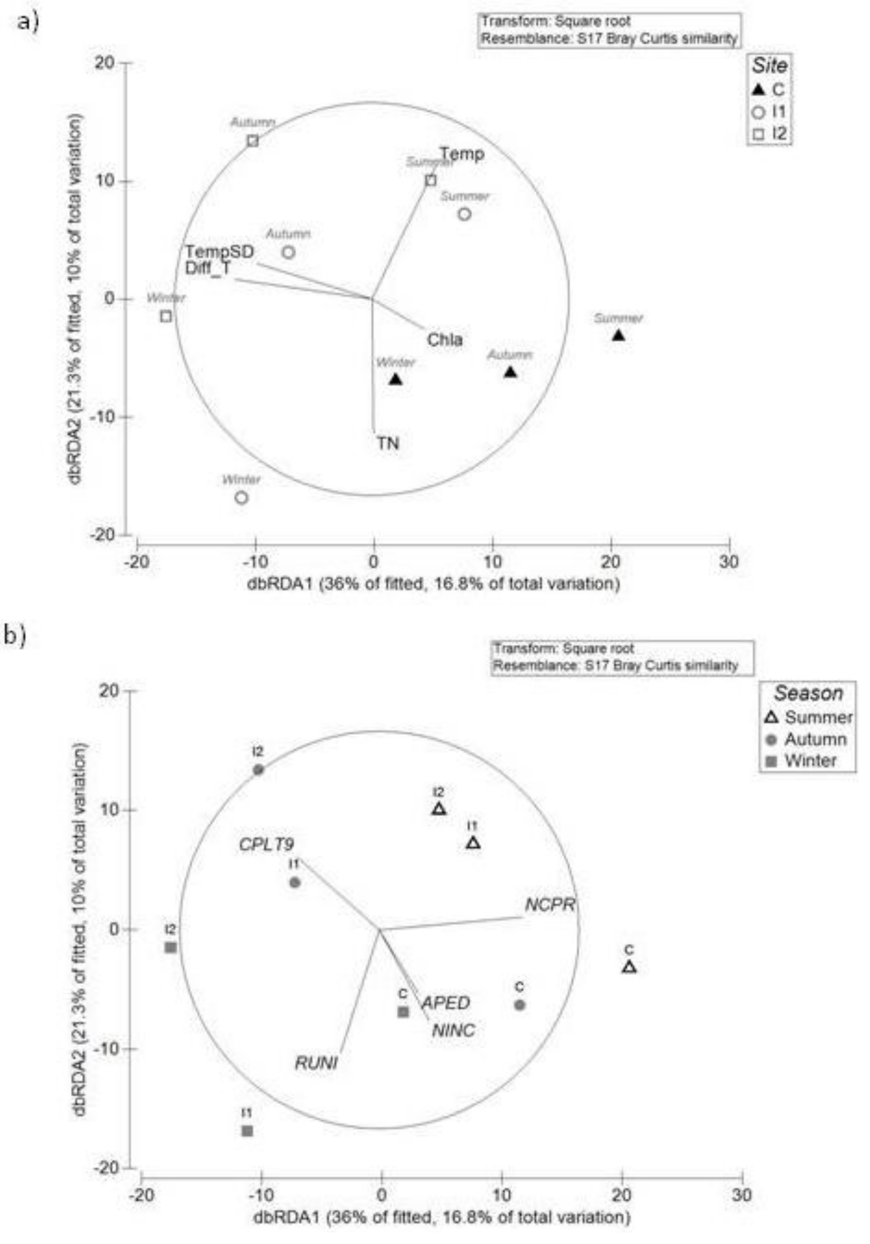

Figure 4. Distance-based Redundancy Analysis (dbRDA) ordination of natural substrate data: a) samples displayed by site and season and vectors showing correlation between explaining variables and dbRDA axes; b) Samples displayed by season and site and vectors showing correlation between the five species with highest contribution to the dissimilarity between control and impacted sites and dbRDA axes. (NCPR= *Navicula capitatoradiata*, APED = *Amphora pediculus*, NINC = *Nitzschia inconspicua*, RUNI = *Reimeria uniseriata*, CPLT9 = *Cocconeis placentula* var. *trilineata).*

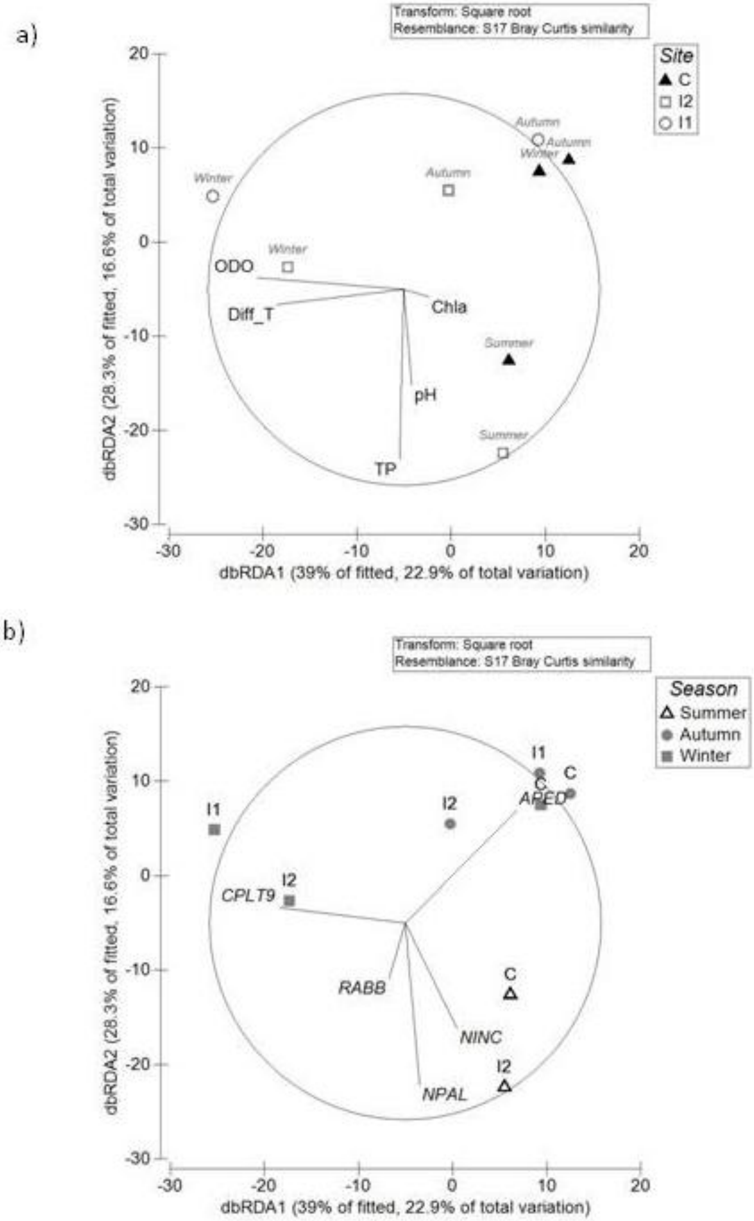

Figure 5. Distance-based Redundancy Analysis (dbRDA) ordination of artificial substrate data: a) samples displayed by site and season and vectors showing correlation between explaining variables and dbRDA axes; b) samples displayed by season and site and vectors showing correlation between the five species with highest contribution to the dissimilarity between control and impacted sites and dbRDA axes. (APED = *Amphora pediculus*, NINC = *Nitzschia inconspicua*, NPAL = *Nitzschia palea*, RABB = *Rhoicosphenia abbreviata*, CPLT9 = *Cocconeis placentula* var. *trilineata*).

The first axis of the dbRDA plots of both natural (Fig. 4) and artificial (Fig. 5) substrates 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) substrates, basically distinguished autumn and winter samples from those of summer and 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 species with the highest contribution to the dissimilarity between control and impacted sites are represented in the dbRDA plots (Figs 4 and 5).

DISCUSSION

The presence of the Nuclear Power Station influences the structure of diatom community at the Ebro River through a sustained increase of water temperature occurring over the last 30 years. The values recorded for environmental variables and the distribution of samples indicated a seasonal variation explained by the natural fluctuation of conductivity, pH, dissolved oxygen and nutrients related with changes in temperature and flow as consequence of the annual cycle and hydrodynamics of the river. However, this process has been altered by a thermal increase consistently greater than 2^{o}C in the river, and the results obtained in this study show the existence of two different diatom assemblages inhabiting in sites before and after the Nuclear Power Station.

Most of environmental variables measured shared the same values or showed a little variation between control and impacted sites; therefore, the differences detected in diatoms assemblages could be mostly attributable to the warming effect, either by its direct influence or by its interaction with other functional process. The sensitivity of diatoms to changes in water temperature is widely recognized (e.g. Moore, 1977a; b; Stevenson & Pan, 1999). Increases in temperature have complex effects, for instance affecting the diffusion rates of chemicals and reducing the amount of oxygen that water may maintain; these changes in the environmental conditions will very likely affect the reproductive rates and metabolism of the algae (Smol & Stoermer, 2010; Stevenson *et al*., 1996) and therefore lead to changes in community structure.

Diatom assemblages were significantly different between control and impacted sites; these differences were mainly due to variation in community composition expressed as species abundances rather than species presence or absence. These changes in abundance could be related to specific physiological responses of species to their optimal temperature ranges, but may also be related to shifts as consequence of interspecies interactions as competition or due to the influence of other environmental variables. Our data does not allow to attribute the observed changes in community structure solely to the temperature alteration, but evidence that warming is a determinant factor influencing or enhancing other factors on the structure of communities.

In this study, the species pool did not show significant variation; we found slightly higher values in species richness and diversity indices at the impacted sites. For algae, it has been documented that diversity increases from 0 to 25 ^{o}C and starts to decrease at temperatures above 30 ^{o}C (Dallas, 2008; Kishi *et al*., 2005; Patrick, 1971), though changes in community structure are usually more evident at temperatures from 25 to 30 ^{o}C rather than < 25 ^{o}C (De Nicola, 1996). During the study period, temperature never exceeded 25 ^{o}C and changes detected in species composition were minor and due to species with low relative abundances (< 5%). Similar results were reported in a study including benthic epilithic communities under thermal influence, where Hillebrand *et al*. (2010) found that elevating the water temperature increased temporal beta-diversity and decreased compositional stability of communities; and instead of changes in species richness, it was observed a change of proportion of species from the same pool. Changes in diatom community structure as consequence of thermal alteration were documented by Squires *et al*. (1979) who found that the algal flora was significantly affected at the section immediately below the discharge point of a power station ; and Vinson & Rushforth (1989) noted that diversity and species richness increased with temperature and maximum values were reached between 25 and 30 ^{o}C, beyond this temperature species diversity decreased; parallel results were also found by Patrick (1969).

The colonization on artificial substrate seemed to be dominated by opportunistic diatom species with fast growth rates such as *Amphora pediculus*, *Nitzschia inconspicua* and *Rhoicosphenia abbreviata*, which can quickly form large blooms and compete with other algal species with slower growth rates, as has been previously highlighted by Snoeijs & Prentice (1989). These species also showed high abundance on natural substrate and in both cases (natural and artificial substrate), dominance was shared with *Cocconeis* spp., which did not show a clear preference between control and impacted sites. These results are opposite to those found by Stevenson (1996) who detected a shift to dominance of *Cocconeis* in warmer waters but this discrepancy could be explained, perhaps, by the results of De Nicola (1996), who noted that *Cocconeis* tended to be more abundant in waters above 25 ^{o}C and as mentioned before, we did not record values exceeding that temperature. Interestingly, although there are some community differences between natural and artificial substrate, both provided essentially the same picture of thermal influence. This agrees with some previous works where again it was found that benthic diatom communities tend to be much more affected by the environmental conditions than by substrate type (Lane *et al*., 2003; Rovira *et al*., 2009; 2012).

Diatom communities proved to be sensitive to water warming even though this alteration did not exceed 3 ^{o}C. The factors that seemed to have most effect on the benthic assemblages inhabiting the area influenced by the Nuclear Power Station, were the seasonal variation and thermal alteration caused by the heated effluent.

Nowadays, aquatic ecosystems are threatened as consequence of greater water demands and climate change, and to ensure their adequate management, it is evident the need to better understand the response of biota to thermal alterations. This is especially important for Mediterranean ecosystems, since this region of the world is going to be among the most impacted ones by climate change, and in particular by global warming (IPCC, 2013). By using the thermal gradient in the Nuclear Power Station flume it is possible to cover part of the range of future scenarios of temperature and therefore, our results could be of interest to predict changes in benthic communities under global warming scenarios. However, it is important to note that this work focused on a local species pool along a period of time when the regional species pool did not change, and it has been pointed out that global warming will lead to turnover also in the regional species pools by actions as emigration or adaptation of species from other regions (Parmesan & Yohe, 2003); thus, changes in local species could also be influenced by changes on regional scales if the temperature increase affect larger areas over longer periods (Hillebrand *et al*., 2010).

We think that the information generated here will contribute to a better understanding of the effects of increasing temperature on the benthic diatom communities of Mediterranean rivers and hence will provide useful baseline data for predicting the effects of global warming under future projected scenarios.

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SUPPLEMENTARY MATERIAL

Appendix 1. – List of diatom taxa found and their relative abundances (%) over the study period at control (C), and impacted sites (I1 and I2).

		Natural Substrate									Artificial substrate							
	Taxa	**Summer**			**Autumn**			**Winter**			**Summer**		**Autumn**			**Winter**		
			I1	**I2**	**C**	**I1**	**I2**	**C**	**I1**	**I2**	**C**	**I2**	**C**	**I1**	**I2**	**C**	**I1**	**I2**
ACON	*Achnanthes conspicua* A. Mayer	0.4	0.1	0.6	0.6	0.1	0.0	0.0	0.0	0.0	0.1	0.4	0.1	0.0	0.0	0.2	0.6	0.3
ADMI	*Achnanthidium minutissimum* (Kützing) Czarnecki	3.4	0.8	1.8	1.5	0.5	0.0	0.7	0.3	3.0	2.1	4.3	7.4	1.5	1.5	1.5	0.0	1.3
ANMN	*Actinocyclus normanii* (Gregory) Hustedt	0.1	0.1	0.0	0.5	0.3	1.1	0.0	0.0	0.3	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
ACYB	*Amphora cymbamphora* Cholnoky	0.0	0.2	0.0	0.0	0.6	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.1
ACOE	*Amphora copulata* (Kützing) Schoeman & Archibald	2.5	6.7	4.8	13.1	10.1	4.5	2.4	4.2	3.8	2.3	0.3	7.2	11.4	5.4	5.5	8.7	4.3
AMID	*Amphora indistincta* Levkov	0.5	5.5	5.5	5.2	1.9	5.3	2.3	3.7	5.8	1.0	0.1	2.1	1.0	0.4	1.5	0.5	0.1
AOVA	*Amphora ovalis* (Kützing) Kützing	0.1	0.8	0.9	1.7	0.7	0.9	0.2	0.8	0.3	0.0	0.1	0.0	0.4	0.8	0.0	5.8	0.6
APED	*Amphora pediculus* (Kützing) Grunow	25.8	22.5	18.8	23.3	10.0	5.5	26.9	21.4	26.4	8.1	2.3	28.3	38.3	8.2	34.9	1.8	6.0
AAMB	*Aulacoseira ambigua* (Grunow) Simonsen	0.0	0.5	0.5	0.0	0.0	0.7	0.4	0.3	1.2	0.0	0.1	0.0	0.0	1.2	0.0	1.4	0.8
AUGR	*Aulacoseira granulata* (Ehrenberg) Simonsen	0.0	0.1	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
BPAR	*Bacillaria paradoxa* Gmelin in Linneaeus	0.1	0.0	0.0	0.0	0.0	0.2	0.0	0.3	0.6	0.0	0.0	0.0	0.0	0.1	0.0	0.6	0.2
CBAC	*Caloneis bacillum* (Grunow) Cleve	0.0	0.1	0.3	0.2	0.3	0.2	0.0	0.2	0.0	0.2	0.1	0.0	0.6	0.6	0.2	0.0	0.0
CPED	*Cocconeis pediculus* Ehrenberg	0.8	4.9	4.8	0.8	0.7	0.7	0.7	0.6	1.5	2.0	3.2	1.1	0.3	1.8	0.9	0.8	3.2
CPLE	*Cocconeis placentula* Ehrenberg var. *euglypta* (Ehrenberg) Grunow	2.5	3.0	3.6	1.6	1.7	6.9	2.8	1.6	4.8	9.4	3.3	7.4	9.4	10.7	6.4	8.0	7.5
CPLI	*Cocconeis placentula* var. *lineata* (Ehrenberg) Van Heurck	3.8	5.5	3.5	2.6	4.1	7.7	2.9	3.7	7.5	8.7	4.5	6.2	3.2	5.8	8.3	9.8	13.6
CPLA	*Cocconeis placentula* Ehrenberg var. *placentula*	2.2	2.1	2.1	0.7	0.6	2.4	1.6	1.5	4.9	7.5	1.9	1.7	1.7	2.3	5.7	5.4	8.9
CPLT9	*Cocconeis placentula* var. *trilineata* (Peragallo & Héribaud) Cleve	0.6	2.4	1.9	0.7	1.4	11.3	1.2	1.9	5.9	3.2	1.9	2.3	1.6	7.5	4.7	7.9	14.2
CSCU	*Cocconeis scutellum* Ehrenberg var. *scutellum*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	2.1	0.5
CAMB	*Craticula ambigua* (Ehrenberg) D.G. Mann	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CDUB	*Cyclostephanos dubius* (Fricke) Round	0.0	0.1	0.2	0.2	0.1	0.4	0.4	0.3	0.2	0.0	0.1	0.3	0.0	0.2	0.2	1.1	0.2
CMEN	*Cyclotella meneghiniana* Kützing	0.9	0.1	0.2	0.0	0.0	0.0	0.0	0.1	0.1	2.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0

COCE	*Cyclotella ocellata* Pantocsek	0.0	0.0	0.0	0.0	0.1	0.2	0.6	0.6	0.4	0.1	0.0	0.0	0.0	0.5	0.2	1.4	2.7
CAFV	*Cymbella affinis* Kützing	0.0	0.0	0.0	0.2	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CLAN	*Cymbella lanceolata* (C. Agardh) Kirchner	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
DCOF	*Diadesmis confervacea* Kützing	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0
DVUL	*Diatoma vulgaris* Bory	0.3	1.2	1.1	1.7	1.0	2.9	2.0	0.3	0.2	0.0	0.4	0.6	0.8	2.0	0.0	0.8	0.9
DOVA	*Diploneis ovalis* (Hilse) Cleve	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.4	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
EARE	*Ellerbeckia arenaria* (Moore) Crawford	0.0	0.3	0.2	0.3	0.2	0.8	0.0	1.0	1.7	0.0	0.1	0.1	0.0	0.7	0.0	0.6	0.4
ENMI	*Encyonema minutum* (Hilse in Rabenhorst) D.G. Mann in Round, Crawford & Mann	0.4	0.8	1.6	0.3	0.1	0.2	0.3	0.0	0.0	0.4	1.1	0.4	0.3	0.3	0.2	0.2	0.0
EPRO	*Encyonema prostratum* (Berkeley) Kützing	0.2	0.3	0.0	0.1	0.8	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.1	0.0	0.1	0.0	0.1
EOMI	*Eolimna minima* (Grunow) Lange-Bertalot	0.0	0.1	0.3	0.4	0.2	0.5	0.0	0.7	0.1	0.2	0.1	0.2	0.5	0.9	0.0	0.0	0.1
ESBM	*Eolimna subminuscula* (Manguin) Moser, Lange-Bertalot & Metzeltin	0.8	0.0	0.5	1.2	0.3	0.1	0.3	1.4	0.1	0.7	0.7	0.9	1.5	0.6	0.9	0.0	0.5
FPYG	*Fallacia pygmaea* (Kützing) Stickle & D.G. Mann in Round, Crawford & Mann	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
FFAS	*Fragilaria fasciculata* (C. Agardh) Lange-Bertalot	0.3	1.5	0.7	0.7	0.9	1.4	0.6	0.6	1.0	0.5	0.4	0.5	0.1	1.1	0.2	1.2	1.6
FVBR	*Frustulia vulgaris* (Thwaites) De Toni	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
GDEC	*Geissleria decussis* (Østrup) Lange-Bertalot & Metzeltin	0.0	0.0	0.1	0.0	0.0	0.0	1.0	0.2	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
GPGR	*Gomphosphenia grovei* (M. Schmidt) Lange-Bertalot	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1
GMIN	*Gomphonema minutum* (C. Agardh) C. Agardh	1.5	2.0	2.0	1.0	1.2	3.2	1.9	0.2	0.6	0.9	7.0	3.2	0.6	1.4	0.8	2.7	2.0
GOLI	*Gomphonema olivaceum* (Hornemann) Brébisson	0.5	0.1	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0
GPAR	*Gomphonema parvulum* (Kützing) Kützing	4.2	0.9	1.1	0.6	0.6	0.0	0.8	0.1	0.3	0.4	5.3	0.0	0.7	0.7	0.4	0.5	0.1
GRHB	*Gomphonema rhombicum* M. Schmidt	0.6	0.2	1.0	0.4	3.5	3.1	2.0	1.5	1.8	0.5	4.9	6.9	1.4	3.5	0.9	2.6	0.9
GTRU	*Gomphonema truncatum* Ehrenberg	0.6	0.1	0.6	0.1	0.1	0.1	0.0	0.0	0.0	0.3	0.1	0.0	0.3	0.0	0.0	0.0	0.0
GYAT	*Gyrosigma attenuatum* (Kützing) Rabenhorst	0.0	1.4	0.2	0.3	0.7	1.2	0.3	1.3	0.8	0.0	0.1	0.1	0.1	0.3	0.1	4.4	0.6
HLMO	*Halamphora montana* (Krasske) Levkov	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
HSAB	*Halamphora sabiniana* (Reimer) Levkov	0.0	0.2	0.0	0.0	0.3	0.3	0.1	0.3	0.2	0.0	0.0	0.1	0.5	0.8	0.0	0.0	0.2
HVEN	*Halamphora veneta* (Kützing) Levkov	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
KCLE	*Karayevia clevei* (Grunow) Round & Bukhtiyarova	0.1	0.0	0.0	0.1	0.3	0.1	0.0	5.8	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
KAPG	*Karayevia ploenensis* (Hustedt) Bukhtiyarova var. *gessneri* (Hustedt) Bukhtiyarova	0.1	0.3	0.2	0.3	0.0	0.3	0.1	0.1	0.1	0.4	0.1	0.3	0.1	0.3	0.6	0.0	0.5
LGOE	*Luticola goeppertiana* (Bleisch) D.G. Mann	0.0	0.0	0.0	0.0	0.0	0.1	0.0	4.0	2.4	0.0	0.0	0.0	0.4	0.0	0.0	0.0	1.1
MPMI	*Mayamaea permitis* (Hustedt) Bruder & Medlin	0.1	0.5	0.0	0.2	0.0	0.1	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

MVAR	*Melosira varians* C. Agardh	0.8	3.7	0.9	5.5	2.6	3.1	3.8	0.6	1.3	2.8	1.6	0.7	0.5	2.8	0.5	3.0	2.3
NANT	*Navicula antonii* Lange-Bertalot	6.5	5.9	6.4	1.7	4.5	5.4	2.2	0.5	0.9	6.8	2.3	0.9	1.1	1.1	3.0	0.7	1.1
NCAP	*Navicula capitata* Ehrenberg	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.2	0.0	0.8	0.0
NCPR	*Navicula capitatoradiata* Germain	12.4	4.9	3.8	0.7	1.6	0.2	2.5	0.0	0.3	1.7	2.1	0.3	0.7	0.6	0.1	0.8	0.7
NCAR	*Navicula cari* Ehrenberg	0.0	0.2	0.0	0.0	0.8	0.1	0.4	0.4	0.0	0.0	0.0	0.1	0.9	1.2	0.0	0.0	0.0
NCTE	*Navicula cryptotenella* Lange-Bertalot	1.4	4.4	5.5	2.0	5.6	2.4	3.1	1.9	2.4	7.2	2.7	1.6	2.1	3.5	7.7	5.3	2.3
NGER	*Navicula germainii* Wallace	0.0	0.0	0.0	0.0	0.0	0.1	0.4	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
NGRE	*Navicula gregaria* Donkin	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	2.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6
NHEL	*Navicula helensis* Schulz	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.0	0.0	0.2
NINO	*Navicula ignota* Krasske	0.4	1.3	1.1	0.0	0.5	0.0	0.3	1.3	0.1	0.7	0.1	0.0	0.6	0.0	0.2	0.0	0.1
NLAN	*Navicula lanceolata* (Agardh) Ehrenberg	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NRCS	*Navicula recens* (Lange-Bertalot) Lange-Bertalot	0.0	0.0	0.0	0.0	3.7	0.0	0.9	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NRCH	*Navicula reichardtiana* Lange-Bertalot in Krammer & Lange-Bertalot	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NTPT	*Navicula tripunctata* (O.F. Müller) Bory	0.0	0.6	1.2	1.8	3.6	5.6	0.5	2.5	0.9	0.0	0.4	0.3	1.1	2.3	0.6	3.7	0.9
NVEN	*Navicula veneta* Kützing	0.0	0.0	0.3	0.0	1.5	0.0	2.8	0.5	1.2	0.0	0.2	0.0	0.1	0.5	0.3	0.8	1.2
NAMP	*Nitzschia amphibia* Grunow	4.1	0.9	2.9	3.2	5.0	3.9	2.6	0.7	2.6	1.1	1.9	5.9	3.4	3.7	1.5	1.2	1.9
NCPL	*Nitzschia capitellata* Hustedt in A. Schmidt & al.	0.3	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.3	1.2	0.0	0.0	0.0	0.1	0.0	0.1
NCOT	*Nitzschia constricta* (Kützing) Ralfs in Pritchard	0.1	0.0	0.0	0.0	0.0	0.0	0.3	0.1	0.0	0.0	0.1	0.0	0.0	0.3	0.0	0.0	0.0
NDEN	*Nitzschia denticula* Grunow in Cleve & Grunow	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.1	0.0	0.0	0.0	0.2	0.0	0.2
NDIS	*Nitzschia dissipata* (Kützing) Grunow	0.3	2.5	3.3	1.7	3.8	0.4	3.4	1.1	0.3	1.2	0.4	0.1	0.9	2.3	2.4	0.3	0.2
NFIL	*Nitzschia filiformis* (W. Smith) Hustedt	0.0	0.2	0.1	0.4	1.0	0.7	0.3	0.7	0.0	0.0	0.4	0.0	0.4	0.2	0.0	0.3	0.0
NIFR	*Nitzschia frustulum* (Kützing) Grunow	0.0	0.0	0.0	0.0	0.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NHEU	*Nitzschia heufleriana* Grunow	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NINC	*Nitzschia inconspicua* Grunow	12.2	1.0	6.1	14.6	12.4	0.1	5.7	11.1	4.5	18.6	23.5	5.6	7.3	10.5	5.2	1.7	4.4
NMIC	*Nitzschia microcephala* Grunow in Cleve & Moller	0.0	0.0	0.0	0.3	0.1	0.0	1.6	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0
NPAL	*Nitzschia palea* (Kützing) W. Smith	4.0	2.2	3.2	0.6	1.0	0.6	1.7	0.5	0.2	4.5	8.5	0.0	0.1	0.5	0.3	1.1	1.5
NREC	*Nitzschia recta* Hantzsch in Rabenhorst	0.0	0.1	0.2	0.0	0.1	0.2	0.0	0.2	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.3	0.0
PLFR	*Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot	0.0	0.0	0.0	0.0	0.6	0.4	0.5	0.1	0.0	0.0	0.0	0.1	0.3	1.9	0.0	0.3	1.5
PTLA	*Planothidium lanceolatum* (Brebisson ex Kützing) Lange-Bertalot	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

PTRO	*Planothidium rostratum* (Østrup) Round & Bukhtiyarova	0.0	0.1	0.1	0.1	0.9	0.2	0.0	0.1	0.1	0.3	0.0	0.1	0.0	0.1	0.0	0.0	0.2
PLEV	*Pleurosira laevis* (Ehrenberg) Compère	0.0	1.0	0.4	0.1	0.4	0.7	1.3	0.5	1.0	0.3	0.1	0.3	0.5	1.1	0.0	3.3	1.2
PSBR	*Pseudostaurosira brevistriata* (Grunow) D.M. Williams & Round	0.0	0.6	0.3	0.4	0.4	0.1	0.3	0.4	0.0	0.1	0.2	0.1	0.0	0.2	0.1	0.3	0.0
RUNI	*Reimeria uniseriata* Sala, Guerrero & Ferrario	2.4	0.4	1.2	2.3	1.5	1.3	5.3	13.5	1.1	0.3	0.1	0.8	0.1	0.1	1.7	0.2	0.2
RABB	*Rhoicosphenia abbreviata* (C. Agardh) Lange-Bertalot	0.3	2.5	2.1	1.2	2.4	4.4	3.0	1.8	1.9	0.7	10.4	5.4	2.0	5.8	1.8	5.8	3.3
SCVE	*Staurosira construens* var. *venter* (Ehrenberg) P.B. Hamilton	0.3	0.4	0.1	2.3	0.3	0.0	0.6	0.1	0.3	0.7	0.0	0.0	0.0	0.1	0.1	0.3	0.7
SBRE	*Surirella brebissonii* Krammer & Lange-Bertalot	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
THLA	*Thalassiosira lacustris* (Grunow) Hasle in Hasle & Fryxell	0.1	0.4	0.2	0.2	0.4	0.5	0.3	0.1	0.0	0.0	0.0	0.1	0.0	0.3	0.1	0.0	0.0
UULN	*Ulnaria ulna* (Nitzsch) Compère	0.7	1.6	1.2	0.3	0.1	0.3	0.6	0.5	0.2	0.3	0.4	0.2	0.3	0.6	0.1	0.5	0.5

Appendix 2. Diatom community descriptive parameters for over the study period at control (C) and impacted sites . Richness (S), Shannon-Wiener's diversity index (H', as $\log_2$) and Pielou's evenness index (J').

	S	H'($\log_e$)	J'
Natural Substrate			
Summer			
C	34	2.58	0.73
I1	40	2.97	0.80
I2	43	3.08	0.82
Autumn			
C	38	2.74	0.75
I1	47	3.10	0.81
I2	40	3.08	0.83
Winter			
C	42	2.91	0.78
I1	42	2.69	0.72
I2	38	2.88	0.79
Artificial Substrate			
Summer			
C	32	2.74	0.79
I2	37	2.67	0.74
Autumn			
C	31	2.55	0.74
I1	37	2.41	0.67
I2	43	3.17	0.84
Winter			
C	30	2.40	0.70
I1	31	3.07	0.89
I2	41	3.05	0.82

Appendix 3. – Similarity Percentages analysis (SIMPER) of diatom taxa showing mean dissimilarity between control and impacted sites and percentages of taxa contribution until reach 50%. a) Natural substrate. b) Artificial substrate.

Natural substrate

Average dissimilarity: 42.22

Species	Control Av.Abund	Impacted Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Amphora pediculus (Kützing) Grunow	4.85	3.87	1.84	1.34	4.37	4.37
Nitzschia inconspicua Grunow	3.1	2.05	1.61	1.35	3.81	8.18
Navicula capitatoradiata Germain	1.93	0.98	1.43	1.28	3.39	11.57
Reimeria uniseriata Sala, Guerrero & Ferrario	1.64	1.37	1.14	1.26	2.69	14.26
Cocconeis placentula var. *trilineata* (Peragallo & Héribaud) Cleve	0.81	1.8	1.07	1.09	2.54	16.8
Amphora copulata (Kützing) Schoeman & Archibald	2.18	2.3	1.03	1.45	2.45	19.25
Navicula tripunctata (O.F. Müller) Bory	0.63	1.32	0.97	1.29	2.3	21.55
Amphora indistincta Levkov	1.49	2.03	0.91	1.35	2.16	23.71
Melosira varians C. Agardh	1.56	1.35	0.89	1.42	2.1	25.81
Nitzschia dissipata (Kützing) Grunow	1.12	1.08	0.87	1.34	2.07	27.88
Cocconeis placentula var. *lineata* (Ehrenberg) Van Heurck	1.63	2.25	0.87	1.44	2.06	29.94
Navicula antonii Lange-Bertalot	1.76	1.78	0.86	1.4	2.04	31.99
Achnanthidium minutissimum (Kützing) Czarnecki	1.2	0.78	0.83	1.39	1.97	33.95
Nitzschia palea (Kützing) W. Smith	1.24	0.93	0.83	1.37	1.96	35.91
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot	1.03	1.43	0.82	1.33	1.94	37.85
Gomphonema parvulum (Kützing) Kützing	1.04	0.56	0.81	1.13	1.91	39.76
Gomphonema rhombicum M. Schmidt	0.79	1.17	0.8	1.3	1.89	41.66
Cocconeis pediculus Ehrenberg	0.73	1.3	0.8	1.23	1.89	43.55
Staurosira construens var. *venter* (Ehrenberg) P.B. Hamilton	0.86	0.26	0.71	1.35	1.69	45.24
Pleurosira laevis (Ehrenberg) Compère	0.33	0.76	0.7	1.8	1.66	46.9
Cocconeis placentula Ehrenberg var. *euglypta* (Ehrenberg) Grunow	1.43	1.8	0.7	1.3	1.65	48.55
Navicula veneta Kützing	0.54	0.49	0.69	1.1	1.64	50.18

Artificial substrate

Average dissimilarity = 39.97

Species	Control Av.Abund	Impacted Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Amphora pediculus (Kützing) Grunow	4.58	2.82	2.75	1.52	6.88	6.88
Nitzschia inconspicua Grunow	2.85	2.69	1.54	1.01	3.86	10.74
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot	1.36	2.16	1.27	1.32	3.17	13.91
Nitzschia palea (Kützing) W. Smith	0.82	1.15	1.17	1.2	2.93	16.85
Cocconeis placentula var. *trilineata* (Peragallo & Héribaud) Cleve	1.78	2.33	1.13	1.33	2.83	19.68
Achnanthidium minutissimum (Kützing) Czarnecki	1.69	1.11	1.05	1.23	2.62	22.29
Amphora copulata (Kützing) Schoeman & Archibald	2.15	2.24	1.04	1.4	2.61	24.91
Gomphonema rhombicum M. Schmidt	1.35	1.49	1	1.33	2.51	27.41
Navicula cryptotenella Lange-Bertalot	2.12	1.7	0.99	1.29	2.48	29.89
Amphora ovalis (Kützing) Kützing	0	0.91	0.95	1.06	2.37	32.27
Navicula antonii Lange-Bertalot	1.64	1.01	0.93	1.09	2.32	34.58
Cocconeis placentula Ehrenberg var. *placentula*	2.11	1.87	0.92	1.44	2.3	36.88
Gomphonema minutum (C. Agardh) C. Agardh	1.11	1.44	0.88	1.18	2.21	39.09
Navicula tripunctata (O.F. Müller) Bory	0.39	1.15	0.87	1.37	2.18	41.27
Cocconeis placentula var. *lineata* (Ehrenberg) Van Heurck	2.75	2.57	0.83	1.3	2.07	43.34
Nitzschia amphibia Grunow	1.51	1.47	0.78	1.43	1.96	45.3
Amphora indistincta Levkov	1.2	0.51	0.78	1.51	1.95	47.26
Gomphonema parvulum (Kützing) Kützing	0.41	0.9	0.78	1.04	1.94	49.2
Pleurosira laevis (Ehrenberg) Compère	0.32	0.99	0.77	1.41	1.93	51.13

Chapter 3

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

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(To be submitted)

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 conducted at sites before and after the effluent and collected from natural and artificial substrata were analyzed and, Non-metrical Multidimensional Scaling (NMDS), Similarity Percentage Analysis (SIMPER) and 1-way Analysis of Similarities (ANOSIM) were performed to assess changes in community structure. 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).

Macroinvertebrates assemblages showed sensitivity to thermal changes both in natural and artificial substrata, even though warming did not exceed 3 ^{o}C. 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. Given that warming conditions in the study area have been permanent during the last 30 years, results could be useful to assess the impacts of global warming on large Mediterranean rivers.

INTRODUCTION

Macroinvertebrates are commonly used in biomonitoring and are considered useful indicators of environmental alterations (Chessman *et al.*, 2007; Hellawell, 1986; Hilsenhoff, 1987; Rosenberg & Resh, 1993). Many groups are ubiquitous, which allows comparison between systems (Allan & Castillo, 2007; Hauer & Lamberti, 2011) and although they are patchily distributed (Covich *et al.*, 1999), 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 history traits and physiological functions of organisms and influences the morphology, physiology, behavior, growth, reproduction, and distribution of species (Kishi *et al.*, 2005; Stanford & Ward, 1983; Sweeney & Vannote, 1984). Some potential effects of increasing temperature on species are higher rates of reproduction and growth, faster development, and shorter generation times (Arnell, 1998; Arthur *et al.*, 1982; Hughes, 2000). 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 (Mulholland *et al.*, 1997). Changes in community structure as response to thermal disturbances have been detected even with a temperature alteration of few degrees centigrade (Kaushal *et al.*, 2010).

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 been shown to alter biological and ecological components of aquatic ecosystems (Caissie, 2006; de Vries *et al.*, 2008; Langford, 1990). 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 ^{o}C (Laws, 1993). However, in Europe, legislation requires that the temperature downstream of the effluent should not increase by more than 3^{o}C (European Union, 2006).

Many authors have studied the ecological effects of temperature in aquatic environments (e.g. de Vries *et al.*, 2008; Hawkes, 1969; Verones *et al.*, 2010), and several have assessed the impact of thermal effluents on benthic communities (e.g. Arthur *et al.*, 1982; Durrett & Pearson, 1975; Langford, 1972; Snoeijs & Prentice, 1989; Wellborn & Robinson, 1996). Some studies have also analyzed the implications of climate change on macroinvertebrate assemblages in Mediterranean climate regions worldwide, and strong effects as consequence of climatic variability are expected (Bonada *et al.*, 2007; Daufresne *et al.*, 2007; Lawrence *et al.*, 2010). 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 Nuclear Power Station). 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.

MATERIALS AND METHODS

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.

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 at 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. 1b). 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 (Prats et al., 2010).

The river at the study area has a total length of 2 km that comprise 1 km before and after the Nuclear Power Station, a mean width of 140 m and the substrate is dominated by gravel. At the lower Ebro River, studies about macroinvertebrate assemblages are scarce (Cid *et al.*, 2008; Muñoz & Prat, 1994).

a)
b)

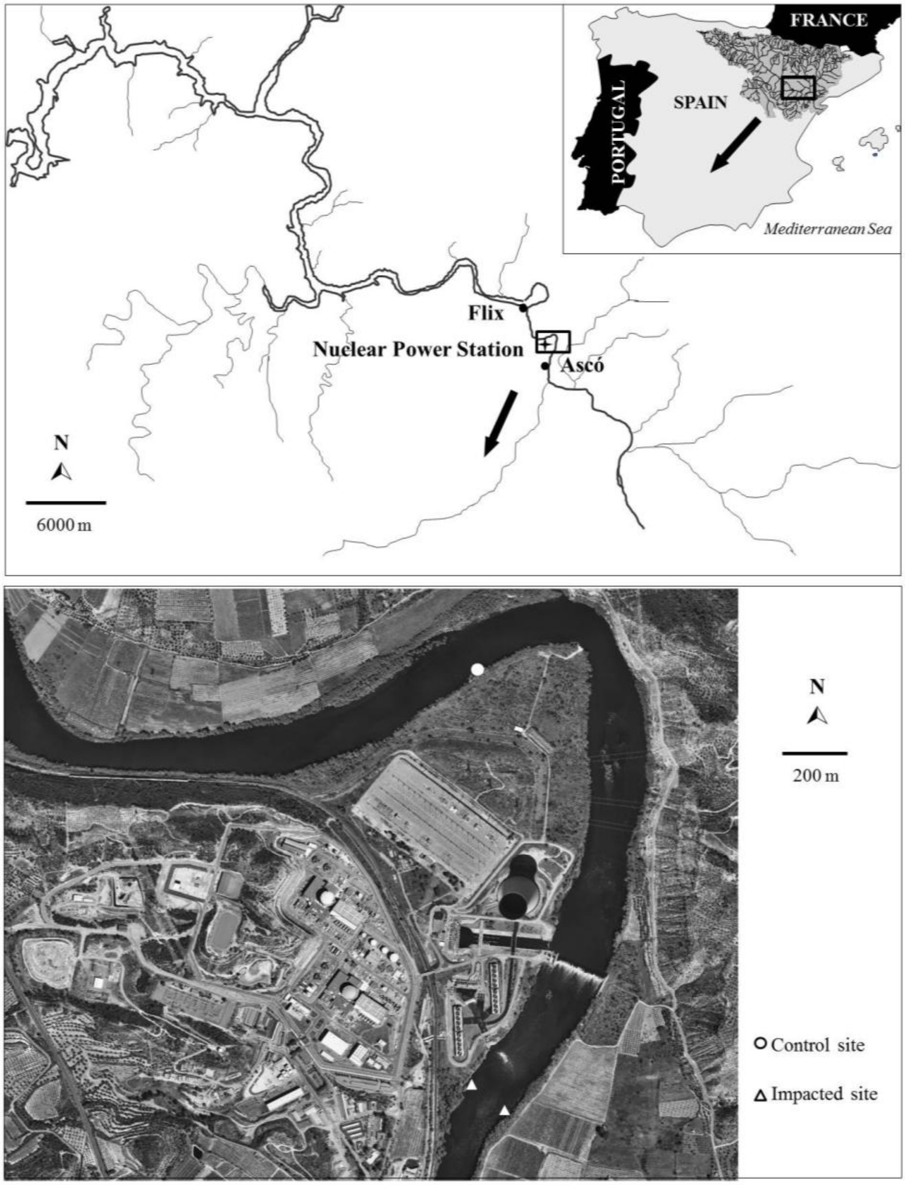


Figure 1. a) Map of the lower Ebro River showing the study area, b) location of sampling sites.

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 (1922) 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 rectangular mesh (5 mm) bag with a bottom cover of 0.25 mm.

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 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. (2000). During the summer campaign the artificial substrates placed on site I1 were not recovered due to vandalism.

For every sampling site and occasion, physicochemical data were recorded. 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 (1977; 1983); and the total chlorophyll concentration was calculate using the colorimetric method (Jeffrey & Humphrey, 1975). At every sampling site, water temperature (°C) was monitored in intervals of 30 minutes during all the study period with a TCtemp1000 Madgetech data logger.

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 with 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) (McArdle & Anderson, 2001) and a set of explanatory variables was identified. The model was visualized through a distance-based redundancy analysis (dbRDA) performed using PRIMER V6 software (Clarke & Gorley, 2006) with the add-on package PERMANOVA+ (Anderson *et al.*, 2008).

RESULTS

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 as consequence of the water heating produced by the cooling system of the Nuclear Power Station (Fig. 2), and was significantly different 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

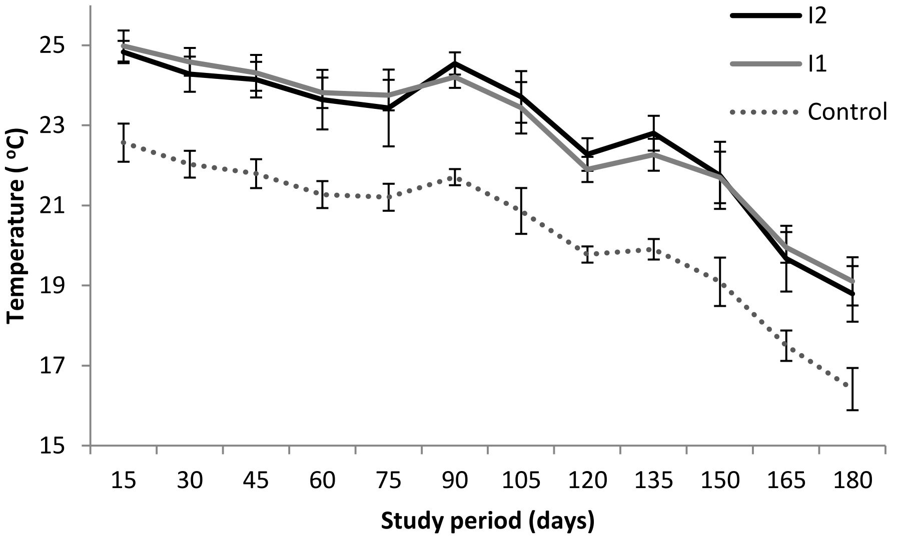


Figure 2. Water temperature recorded over the study period at control (C) and impacted (I1, I2) sites.

Macroinvertebrate assemblages

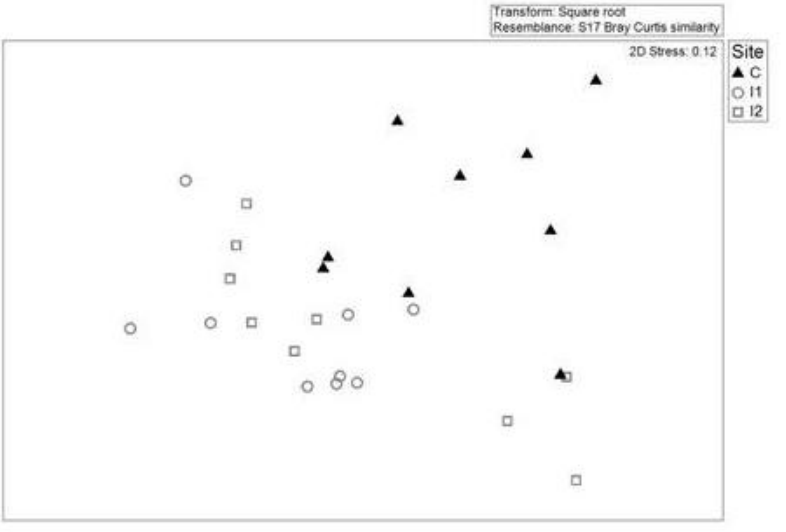
During the study period a total of 37 and 46 taxa were found in natural and artificial substrate, respectively (Supplementary material in Appendix 1). Arthropoda was the dominant phylum and accounted for 41.73% of the total abundance. The most abundant taxa found in natural substrate were Nemertea (17.58%), *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).

a)



b)

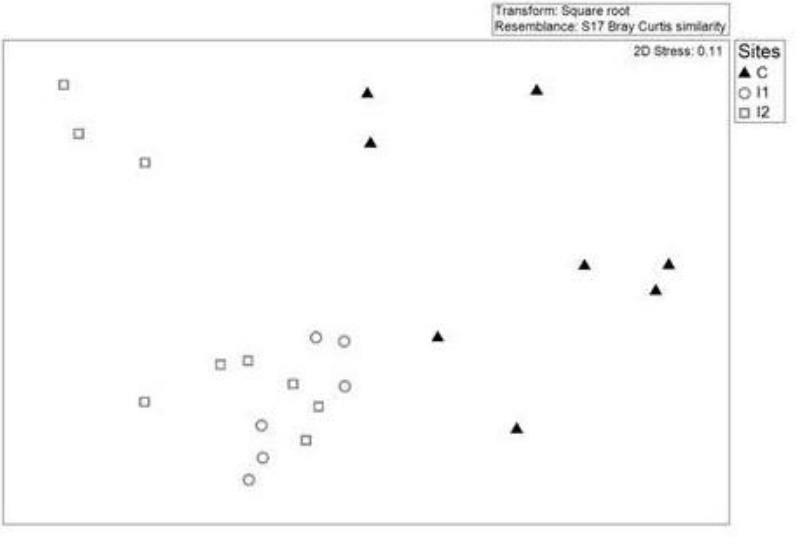


Figure 3. Two dimensional NMDS plots based on Bray-Curtis similarities of square-root transformed macroinvertebrate abundance data. a) Natural substrate ordination. b) Artificial substrate ordination.

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 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*.

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.

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).

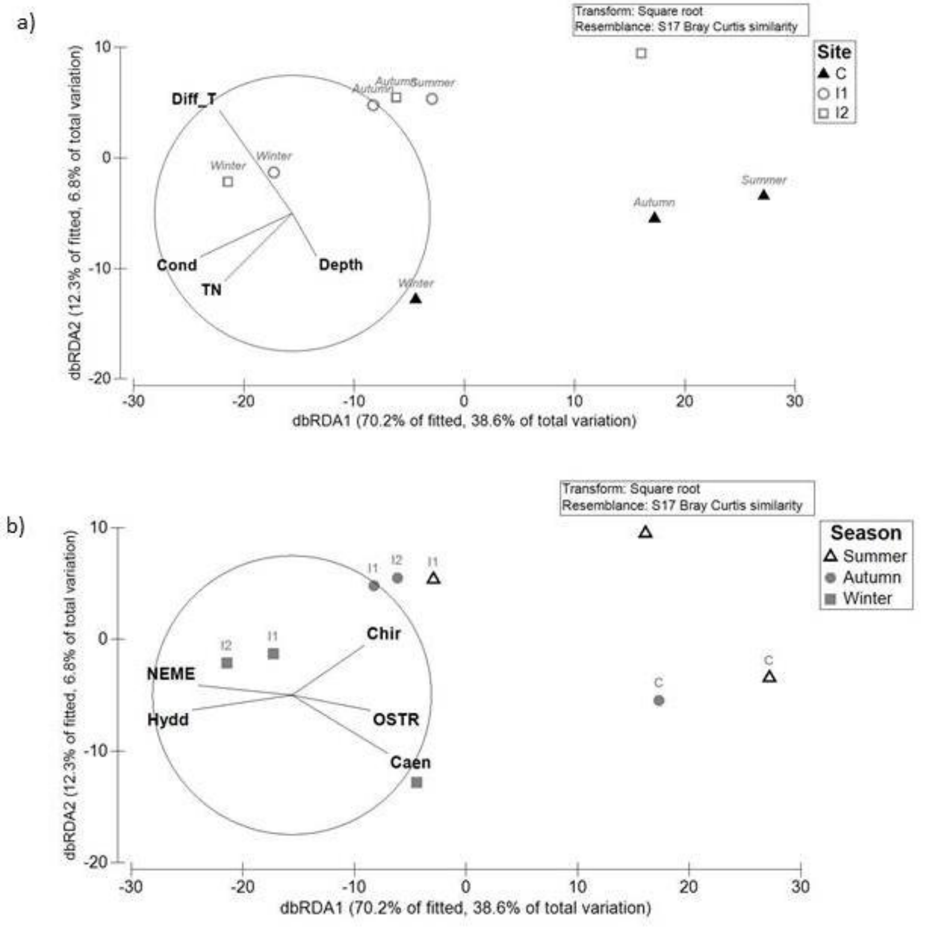


Figure 4. Distance-based Redundancy Analysis (dbRDA) ordination of natural substrate data: a) samples displayed by site and season and vectors showing correlation between explaining variables and dbRDA axes; b) Samples displayed by season and site and vectors showing correlation between the five taxa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

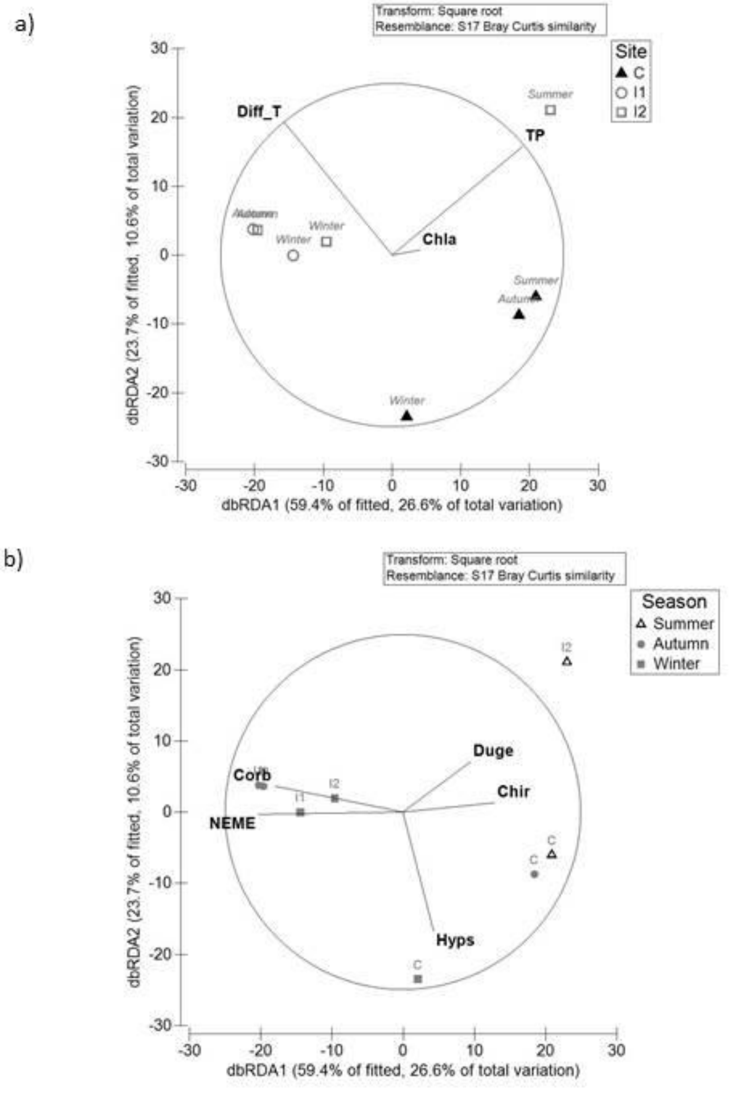


Figure 5. Distance-based Redundancy Analysis (dbRDA) ordination of artificial substrate data: a) samples displayed by site and season and vectors showing correlation between explaining variables and dbRDA axes; b) samples displayed by season and site and vectors showing correlation between the five taxa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

DISCUSSION

The macroinvertebrate community of the lower Ebro River has been influenced by a sustained increase of 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 ^{o}C. Most of the measured environmental variables did not differed 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 process. These results are in agreement with other similar studies analyzing the consequences of thermal alteration on benthic assemblages (Gibbons & Sharitz, 1981; Howell & Gentry, 1974; Langford, 1990; Wellborn & Robinson, 1996). Although thermal pollution has often adverse effects on macroinvertebrates (Alston *et al.*, 1978; Durrett & Pearson, 1975; Howell & Gentry, 1974; Wellborn & Robinson, 1996), increases in abundances and higher values of richness have been also detected (Dahlberg & Conyers, 1974; Dusoge & Wisniewski, 1976; Gibbons, 1976; Poff & Matthews, 1986), 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 (e.g. Gibbons & Sharitz, 1981; Howell & Gentry, 1974; Wellborn & Robinson, 1996), 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

(Alcaraz *et al.*, 2011; Ibáñez *et al.*, 2012; Suárez-Serrano *et al.*, 2010). 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 (Langford, 1983; Lessard & Hayes, 2003).

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 (Leuven *et al.*, 2007), 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 (Wijnhoven *et al.*, 2003). 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 (Durance & Ormerod, 2010; Hauer & Lamberti, 2011) and in marine intertidal systems (Sagarin *et al.*, 1999), 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 (e.g. Langford, 1990; Simard *et al.*, 2012).

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 (e.g. Benzie, 1984; Khalaf & Tachet, 1980).

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 (Bonada *et al.*, 2007; Daufresne *et al.*, 2007; Lawrence *et al.*, 2010), where significant responses in benthic communities related to long-term temperature increases have been identified. In one of them, Bonada et al. (2007), 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. (2007) observed gradual changes in macroinvertebrate community structure under climate change conditions, attributable to high temperatures associated with decreasing oxygen contents; and Lawrence et al. (2010) 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 (IPCC, 2013), the challenge of understanding the consequences of warming on biodiversity remains as a main research subject in this region. A substantial warming (≈1.5°C in winter and ≈2°C in summer) might affect the Mediterranean region in the 2021-2050 period compared to the reference period (1961-1990), in an A1B emission scenario (IPCC, 2013). Consequently, the thermal gradient caused by the Nuclear Power Station provides an excellent opportunity to predict changes in benthic communities under global warming scenarios, allowing isolating the temperature as an independent variable and thus minimizing the difficult that usually have field experimentation on warming effects.

However, it is difficult to predict the 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 (Hawkins *et al.*, 1997; Pritchard *et al.*, 1996).

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

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SUPPLEMENTARY MATERIAL

Appendix 1. – List of macroinvertebrate taxa found and their relative abundances (%) over the study period at control (C), and impacted sites (I1 and I2).

	Natural Substrate									Artificial substrate							
	Summer			Autumn			Winter			Summer		Autumn			Winter		
	C	**I1**	**I2**	**C**	**I1**	**I2**	**C**	**I1**	**I2**	**C**	**I2**	**C**	**I1**	**I2**	**C**	**I1**	**I2**
PHYLUM ANNELIDA																	
Class Clitellata																	
Erpobdellidae	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Erpobdella	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.3	0.0	0.0	0.1	0.1
Helobdella stagnalis	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	3.0	0.0	0.2	1.8
Piscicolidae	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.8
Oligochaeta	2.4	0.7	0.4	0.1	1.8	3.7	14.3	1.2	2.6	0.0	0.0	0.0	3.8	8.1	0.0	1.0	8.5
PHYLUM ARTHROPODA																	
Class Arachnida																	
Hydracarina	2.6	2.1	2.7	2.2	2.8	4.8	1.4	13.1	1.6	0.0	2.5	0.6	0.2	0.2	3.0	1.7	0.0
Class Branchiopoda																	
Cladocera	0.0	0.0	0.0	3.6	3.5	3.1	2.1	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1
Class Entognatha																	
Collembola	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1
Class Maxillopoda																	
Copepoda	1.4	6.9	0.7	9.1	22.3	13.9	13.1	10.7	10.6	10.3	0.2	0.1	0.0	0.1	0.0	0.1	1.0

Class Insecta																	
Atrichops crassipes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Baetidae	0.3	10.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.7	0.0	0.0	0.0	0.0	0.0	0.1
Beraeidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0
Caenis	33.9	0.3	2.2	33.3	1.1	0.7	3.1	2.4	1.3	0.0	1.2	0.8	0.1	1.6	2.0	0.9	1.0
Ceratopogonidae	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.1	0.6	0.0	0.1	1.3	0.0	0.0	0.0
Chironomidae	7.0	6.6	51.6	25.2	9.2	4.7	5.3	0.0	3.0	0.4	21.0	68.6	2.5	2.5	2.3	0.6	2.0
Coenagrion	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.0	0.7	1.6
Dryops	0.0	0.0	0.1	0.1	1.8	0.0	0.0	0.0	2.8	0.0	0.5	0.0	0.8	0.6	0.0	0.5	0.8
Ecnomidae	0.0	0.0	0.0	0.1	0.3	0.0	0.0	1.2	0.0	0.0	0.0	1.0	1.1	9.1	1.0	1.2	1.0
Elmidae	0.0	0.0	0.5	0.0	0.2	0.8	0.0	0.0	0.4	0.0	0.3	0.0	0.0	1.9	0.0	0.0	0.5
Gerridae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Hydrophilidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.4	0.0	0.0	0.0	0.0	0.0	0.0
Hydropsychidae	0.0	0.0	0.8	0.9	0.0	0.3	6.8	0.0	0.0	0.0	0.8	6.9	0.1	0.1	35.9	1.3	0.5
Hydroptila	0.0	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.1	0.0	0.0	0.0	0.0
Hydroptilidae	0.0	0.0	2.5	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.3	0.0	0.0
Orthotrichia	0.3	0.9	0.6	0.1	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.3	2.1	0.8
Leptoceridae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Libellula	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Limoniidae	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Muscidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Naucoridae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0
Platycnemis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.2	2.5	0.0	0.0	1.1

Psychomyiidae	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rhagionidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
Tipulidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0
Class Malacostraca																	
Asellus aquaticus	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.5	0.1	0.3	0.6
Atyaephyra desmarestii	0.0	0.0	0.3	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.2
Procamburus clarkii	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gammaridae	0.0	0.2	4.9	0.0	0.0	0.3	6.8	0.0	0.6	0.2	5.6	2.6	0.3	3.7	16.7	1.1	2.5
Class Ostracoda	30.0	7.1	3.8	2.9	3.7	1.7	3.6	1.4	4.9	9.4	0.5	0.1	1.0	0.8	0.6	1.6	1.6
PHYLUM CNIDARIA																	
Hydridae	0.1	0.3	0.1	0.2	0.3	12.1	2.3	14.1	17.7	0.4	0.0	4.0	4.4	5.9	3.4	9.2	2.8
PHYLUM MOLLUSCA																	
Class Bivalvia																	
Corbicula	9.3	19.1	4.6	12.0	15.4	27.4	7.5	14.4	19.9	1.4	2.2	0.0	38.1	8.1	5.9	6.9	12.6
Dreissena polymorpha	0.7	3.5	0.3	0.6	1.2	0.7	0.4	0.7	0.0	3.4	0.0	0.0	0.4	0.2	0.6	0.5	0.3
Class Gastropoda																	
Radix	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Theodoxus fluviatilis	0.5	0.4	10.3	0.0	0.0	8.1	0.0	0.0	6.6	0.8	1.2	0.0	0.6	12.7	0.7	0.2	7.8
Physella acuta	0.0	1.3	0.0	0.0	0.0	0.2	0.0	0.0	0.4	0.3	1.6	0.0	2.0	2.5	0.1	0.7	3.2
PHYLUM NEMATODA	0.4	0.7	0.1	0.3	1.7	1.3	5.8	0.7	2.5	0.3	0.2	0.0	1.4	0.4	0.2	0.9	0.8
PHYLUM NEMERTEA	4.6	29.0	7.8	4.6	32.0	10.8	21.2	27.8	20.4	3.7	2.9	2.8	24.4	11.5	10.5	39.8	17.1
PHYLUM PLATYHELMINTHES																	
Dugesiidae	6.3	10.5	4.1	4.2	2.7	5.1	4.9	11.0	3.7	67.0	42.5	11.8	18.0	22.2	16.1	28.2	28.3

Appendix 2. – Similarity Percentages analysis (SIMPER) of macroinvertebrate taxa showing mean dissimilarity between control (C) and impacted (I) sites and percentages of taxa contribution up to 50% (accumulated). a) Natural substrate. b) Artificial substrate.

a) Natural substrate

Average dissimilarity: 68.37

Species	Control Av.Abund	Impacted Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Caenis	4.27	0.79	8.43	1.53	22.54	22.54
Chironomidae	2.84	2.7	5.67	1.13	15.16	37.7
Nemertea	2.76	4.38	5.32	1.45	14.23	51.93

b) Artificial substrate

Average dissimilarity = 39.97

Species	Control Av.Abund	Impacted Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Chironomidae	1.15	4.98	7.05	1.39	12.63	12.63
Corbicula	3.16	1.14	3.95	1.29	7.07	19.7
Hydropsychidae	0.46	2.7	3.93	1.17	7.04	26.73
Nemertea	4.1	2.06	3.73	1.53	6.68	33.42
Dugesiidae	5.53	4.66	3.3	1.19	5.91	39.32
Gammaridae	0.99	2.47	2.69	1.41	4.81	44.13
Oligochaeta	1.71	0	2.66	1.51	4.77	48.9
Theodoxus fluviatilis	1.63	0.57	2.13	1.21	3.82	52.71

GENERAL DISCUSSION

This thesis provides evidence that benthic communities at the lower Ebro River have been influenced by alterations in the natural river flow regime due to the presence of the flow regulation system, and in the temperature regime due to a sustained heating of river water over the last 30 years (caused by the presence of the Ascó nuclear power station). In both cases, changes detected in benthic community structure were mainly related with these anthropogenic stressors.

Present hydrodynamic conditions at the lower Ebro correspond to a highly regulated river, where the structure and diversity of benthic communities are affected by different alterations directly or indirectly related to damming, including longitudinal habitat fragmentation and homogenization, water quality impairment, thermal pollution and the presence of invasive species (Cid *et al*., 2010; Ibáñez *et al*., 2008, 2012; Prats *et al*., 2010). In this context, benthic communities (macroinvertebrates and diatoms) have been recognized as useful indicators of hydromorphological and chemical alterations, and biomonitoring protocols based on benthic communities are widely used for running waters worldwide (e.g Alba Tercedor et al., 2002; Furse, 2006; Kelly & Whitton, 1998; Metcalfe, 1989; Quevauviller *et al*., 2008).

In **Chapter 1**, the entire hydrogeomorphic variability of the lower Ebro River was considered in order to assess the structure and composition of benthic macroinvertebrates and diatoms as a function of anthropogenic alterations along a spatial gradient (i.e. distance to dams). Some spatial and seasonal differences were detected and two different communities of macroinvertebrates as function of distance to the dams were identified. The assemblage inhabiting the uppermost sampling section located close to the Flix dam was significantly different to the rest found at sections downstream. The flow regulation system was identified as the main factor influencing structure and distribution of benthic communities. However, in the upper section water temperature was strongly modified by the Ascó nuclear power station, and the impact of thermal pollution on benthic communities was approached in **Chapters 2** and **3.**

Chapter 1 is one of the few studies including a complete characterization of benthic communities of macroinvertebrates and diatoms in a large Mediterranean river using a methodology that covers all the morphological variability. However, our results suggest that natural hydrogeomorphic variability at reach scale was likely not a significant factor determining the structure of benthic communities in the lower Ebro River. We found that the community structure of macroinvertebrates only was significantly different in the section closer to the flow regulation system and results suggest that this was mostly due to the impact of dams rather than to the particular hydrogeomorphic conditions of the study area. However, it has to be taken into account that river flow regulation is also responsible of changes in the hydromorphology of downstream reaches. Similar conclusions are found in other studies in large Mediterranean rivers (e.g. Bournaud *et al*., 1996; Chatzinikolaou *et al*., 2006; Marchetti *et al*., 2011; Muñoz & Prat, 1996) pointing the flow regulation system as one of the main anthropogenic alterations on macroinvertebrate communities. In fact, environmental flows in dammed rivers are now considered as a key issue to achieve the good ecological status of superficial water bodies as required by the Water Framework Directive of the European Union (European Commission, 2012).

Results obtained with benthic diatoms do not detect significant differences among sections along the study area; evidencing that macroinvertebrates and diatoms respond rather differently to anthropogenic stressors, being macroinvertebrates more sensitive to physical changes in river habitat, while diatoms are more sensitive to water quality alterations (Hering *et al*., 2006; Pace *et al*., 2012; Soininen & Könönen, 2004; Triest *et al*., 2001). The structure and composition of diatom assemblages were clearly influenced by seasonal variation, and this temporal variability was related to fluctuating along-year conditions of the lower Ebro River which involves variation in sunlight intensity, changes in water temperature and differences in nutrient concentrations, as well as changes in flow pattern, which appear to be a major factor controlling the observed seasonal changes, in agreement with the findings of Boix *et al*. (2010), Martínez de Fabricius *et al*. (2003) and Tang *et al*. (2013).

Both biological groups (macroinvertebrates and diatoms) demonstrated to be complementary for biomonitoring proposes and in both cases the lowest scores of ecological status were found at the section closest to the flow regulation system. However, when the entire study area is considered, ecological status including other biological indicators also evidence affectation downstream, for instance, the fish community of the lower Ebro River is strongly dominated by invasive species which are favored by dam regulation and river flow reduction (Caiola *et al*., 2014), and the ecological status of the lower Ebro River according to this indicator varies between “poor” and “bad” (Sostoa *et al*., 2010).

The discrepancy between the ecological status scores when assessed with fish and benthic communities is due to the methodology used in the development of the biotic indices. Although the Water Framework Directive asks for an holistic assessment of the ecological status that take into account different sources of human impacts and pressures, the truth is that most macroinvertebrate and diatoms based assessment methods were developed only measuring the response of biological communities to the water quality, whereas the fish indices such as the one developed for Catalan rivers, IBICAT (Sostoa et al., 2010), take into account a wider range of human induced stressors (water quality, hydromorphology, land use, etc.). This means that the fish based indices assess the ecological status in a more realistic way regarding the Water Framework Directive criteria.

In **Chapter 1**, it was found that the community structure of macroinvertebrates is influenced by hydromorphologic aspects, such as the substrate and the distance to the nearest dam (a proxy of flow dynamics). This means that macroinvertebrates are potentially good indicators of other human induced stressors rather than only water quality. Therefore, the development of new macroinvertebrate based biotic indices, or the adaptation of existing ones, following the Water Framework Directive holistic criteria is possible and even desirable.

In **Chapters 2** and **3**, the influence of a thermal discharge caused by the cooling system of the Ascó nuclear power station on benthic communities (diatoms and macroinvertebrates) was assessed. In the study area, the river has been subjecting to a sustained heating during the last 30 years and therefore provides an excellent opportunity for assessing the long-term effects of water warming on benthic communities. Results proved that these two biological groups are sensitive to the sustained water temperature increase even though this alteration did not exceed 3 ^{o}C. We found that aquatic fauna was significantly affected at the section under the influence of the thermal discharge produced by the Ascó nuclear power station, which is consistent with several studies about the consequences of thermal alteration on benthic assemblages (e.g. Gibbons & Sharitz, 1981; Langford, 1990; Squires *et al*., 1979; Vinson & Rushforth, 1989; Wellborn & Robinson, 1996; De Nicola, 1996; Kishi *et al*. 2005). Most of the measured environmental variables showed very similar values or little variation between control and impacted sites; therefore, the differences detected in macroinvertebrate and diatom assemblages were mostly attributable to the warming effect, either by its direct influence or by its interaction with other environmental variables or functional process.

In **Chapter 2**, we found differences in diatoms assemblages, mainly attributable to variation in community composition expressed as species abundances rather than species presence or absence; these changes in abundance could be related to specific physiological responses of species to their optimal temperature ranges, but may also be related to shifts as consequence of interspecies interactions such as competition, or due to the influence of other environmental variables. Increases in temperature have complex effects, for instance affecting the diffusion rates of chemicals and reducing the amount of oxygen that water may maintain; these changes in the environmental conditions will very likely affect the reproductive rates and metabolism of the algae (Smol & Stoermer, 2010; Stevenson *et al*., 1996) and therefore lead to changes in community structure. Our data does not allow to attribute the observed changes in diatom community structure solely to the temperature alteration, but evidence that warming is a determinant factor influencing or enhancing other factors on the structure of communities.

The colonization on artificial substrata seemed to be dominated by opportunistic diatom species with fast growth rates such as *Amphora pediculus*, *Nitzschia inconspicua* and *Rhoicosphenia abbreviata*, which can quickly form large blooms and compete with other algal species with slower growth rates, as has been previously highlighted by Snoeijs & Prentice (1989). These species also showed high abundance on natural substrata and in both cases (natural and artificial substrate), dominance was shared with *Cocconeis* spp.

In **Chapter 3**, we also found a macroinvertebrate assemblage significantly affected under the influence of warming produced by the nuclear power station, which is consistent with several studies about the consequences of thermal alteration on benthic assemblages (Gibbons & Sharitz, 1981; Howell & Gentry, 1974; Langford, 1990; Wellborn & Robinson, 1996).

The structure of the macroinvertebrate community inhabiting the study area was characterized in terms of abundance by tolerant taxa such as Oligochaeta, Chironomidae, Dugesiidae, Nemertea and *Corbicula*, reflecting the habitat degradation of the lower Ebro River caused by decades of alteration in nutrient levels, the presence of large dams upstream and the existence of polluted sediments at the Flix reservoir (Ibáñez *et al*., 2012; Suárez-Serrano *et al*., 2010).

We found that nonnative taxa as *Corbicula* and *Physella* seems to thrive in the heated water, and abundances recorded at impacted sites were higher than values obtained at control site. Similar results have also been previously documented in studies conducted in artificial thermal plumes (e.g. Langford, 1990; Simard *et al*., 2012).

Natural and artificial substrata provided essentially the same picture of thermal influence, which agrees with several works where artificial substrata have proven their usefulness for the assessment of riverine ecosystems (e.g. Benzie, 1984; Khalaf & Tachet, 1980).

Significant changes in macroinvertebrate community composition were detected between control and impact sites even when no differences were distinguished in diversity metrics. These changes identified in macroinvertebrate community structure after a prolonged exposition to higher water temperature are in concordance with other studies carried out in rivers of Mediterranean climate regions (Bonada *et al*., 2007; Daufresne *et al*., 2007; Lawrence *et al*., 2010), which is considered to be a world region among the most impacted ones by climate change, and in particular by global warming (IPCC, 2013; 2014).

As the range of temperature registered at the study area are within the bounds of IPCC climate change predictions for Mediterranean region, the information generated in this study can be useful for a better understanding of warming effects on benthic communities of large Mediterranean rivers and hence provide useful baseline data for assessing the effects of global warming under projected scenarios.

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