Universitat de Girona

AQUATIC COMMUNITY PATTERNS ACROSS ENVIRONMENTAL GRADIENTS IN A MEDITERRANEAN FLOODPLAIN AND THEIR APPLICATION TO ECOSYSTEM RESTORATION

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Aquatic community patterns across environmental gradients in a Mediterranean floodplain and their application to ecosystem restoration

Ph-D dissertation
Belinda Gallardo
Zaragoza-Girona, 2009

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Aquatic community patterns across environmental gradients in a Mediterranean floodplain and their application to ecosystem restoration

Memoria presentada por Belinda Gallardo Armas para optar al
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Para Nacho, que me ha aguantado

cuatro años de carrera, cuatro años y pico de tesis,

y los que nos quedan…

Abstract

In Europe and North America, up to 90% of floodplains have become extensively regulated, occupied, and polluted, with important consequences in the floodplain structure and functionality. The response of this ecosystem to human alteration is complex and its study is of great relevance in the development of management plans to effectively counteract the influence of negative human impacts. To address these needs, the present study provides a solid background for river-floodplain restoration through the investigation of the response of aquatic communities, especially macroinvertebrates, to changes in hydrological connectivity, environmental factors and human pressure across the floodplain of a Mediterranean system, the Middle Ebro River (NE Spain).

Our results showed how the lateral hydrological connectivity is the key factor structuring the habitats and aquatic communities of the Middle Ebro floodplain. This hydrological connectivity was related to a wide variety of environmental processes affecting directly and indirectly the presence of aquatic organisms, from habitat re-organization to nutrient transport. Moreover, hydrological connectivity explained at least one third of the macroinvertebrate species and trait composition, and more than half of the observed variability in the abundance and richness of macroinvertebrates, zooplankton and phytoplankton. Turbidity, salinity and nutrient status were secondary drivers, although essential for understanding the adaptation of aquatic communities to the floodplain environmental variability. On the other hand, the present study allowed identifying surrogates of hydrological fluctuations that were useful to assess the abundance, richness and composition of aquatic communities. For instance, the macroinvertebrate community depended on the duration of the connections established between each wetland and the river channel, whereas organisms with short life-cycles, like phytoplankton and zooplankton, responded better to water-level fluctuations.

The combination of hydrological connectivity and environmental variability create a spatio-temporal template for the aquatic community that enables predictions about the taxonomic and functional patterns more likely to occur under particular conditions. According to the Ebro template (a) frequently flooded habitats, as the river channel and adjacent wetlands, were dominated by insects and aquatic worms that showed disturbance resistance (e.g., small body-shape, protection of eggs) and resilience (e.g., short life-span, asexual reproduction) abilities, (b) by contrast, confined wetlands that are rarely flooded, including oxbow lakes, were dominated by non-insect taxa, as crustaceans and gastropods, showing the ability to interact with other organisms (e.g., large size, sexual reproduction), as well as to effectively use habitats and resources (e.g., diverse locomotion and feeding strategies), (c) the combination of habitat heterogeneity and nutrient availability under intermediate hydrological conditions resulted in highest macroinvertebrate species and trait diversity and, (d) the abundance and richness of three different groups of organisms (zooplankton, phytoplankton and macroinvertebrates) peaked at increasing levels of hydrological connectivity (flooded during 5, 30 and 40 days yr^{-1} respectively).

In the Middle Ebro River, progressive regulation of river flow has altered the natural river-floodplain interaction. Consequently, there are very few possibilities of new wetlands creation, while the diversity and functionality of the remaining ones are threatened by the limited hydrological connectivity. The objective of river-floodplain restoration in the Middle Ebro River should thus consist in re-establishing a wide range of wetland types in accordance with the river-floodplain potential for ecological sustainability. In order to achieve this, a number of actions can be positively performed, such as the modification of embankments, the re-connexion of isolated floodplain habitats, the creation of new wetlands, the recovery of unproductive areas of the floodplain and the control of the water quality at a catchment scale.

Resumen (Castellano)

Aproximadamente el 90% de las llanuras de inundación de grandes ríos europeos y norte-americanos se han visto fuertemente reguladas, ocupadas y contaminadas en las últimas décadas, con importantes consecuencias sobre la estructura y funcionalidad del ecosistema fluvial. La respuesta de este ecosistema a dichos cambios es compleja y su estudio resulta imprescindible para la efectiva implementación de planes de gestión que contrarresten el impacto negativo de las actividades antrópicas. La presente tesis ofrece una base de conocimiento para la restauración de ecosistemas fluviales a través de la respuesta de las comunidades acuáticas, especialmente macroinvertebrados, a cambios en las condiciones hídricas, factores ambientales y presión antrópica a través del gradiente hídrico lateral que se establece de forma natural entre el río y su llanura de inundación. Esta tesis se enmarca en el ámbito de un ecosistema mediterráneo, el río Ebro en su tramo medio (NE, España).

Los resultados obtenidos muestran como la conectividad hídrica lateral es el factor principal que determina la estructura de hábitats y comunidades acuáticas en el Ebro Medio. Esta conectividad está relacionada con un amplio abanico de procesos que afectan directa o indirectamente la presencia de organismos acuáticos, desde la reorganización de hábitats al transporte de nutrientes. Asimismo, la conectividad hídrica es responsable de al menos un tercio de la composición de especies y rasgos biológicos de la comunidad de macroinvertebrados, y de más de la mitad de la variabilidad observada en la abundancia y riqueza de macroinvertebrados, zooplancton y fitoplancton. Otros factores importantes e imprescindibles para comprender la adaptación de las comunidades acuáticas a la heterogeneidad natural de la llanura de inundación, incluyen características físico-químicas como la turbidez, salinidad y estado trófico. Por otro lado, el presente estudio permite identificar un conjunto de variables relacionadas con las fluctuaciones hídricas con las que estudiar la respuesta de las comunidades acuáticas. Por ejemplo, la composición, riqueza y abundancia de macroinvertebrados depende en gran medida de la duración de la conexión hídrica que se establece entre su hábitat y el cauce fluvial, mientras que organismos con ciclos vitales más cortos, como zooplancton y fitoplancton, responden mejor a variaciones en el nivel del agua.

La combinación de conectividad hídrica y características físico-químicas conforma un marco espacio-temporal para el desarrollo de las comunidades acuáticas, que permite realizar predicciones respecto a las características taxonómicas y funcionales de las comunidades acuáticas. De acuerdo con el marco ecológico desarrollado para el Ebro Medio (a) las comunidades acuáticas en ambientes inundados con frecuencia, como el río y brazos secundarios, están dominadas por larvas de insectos y oligoquetos los cuales muestran una gran habilidad para resistir (ej., tamaño pequeño, protección de los huevos) y recuperarse (ej., ciclo de vida corto, reproducción asexual) tras una perturbación, (b) por el contrario, los humedales que se inundan raramente, como galachos confinados, están dominados por crustáceos y gasterópodos que muestran una gran capacidad de interacción con otros organismos (ej., gran tamaño, reproducción sexual) y de utilizar eficientemente los recursos a su alcance (ej., gran diversidad de formas de locomoción y alimentación), (c) en condiciones de conectividad hídrica intermedia, la combinación de heterogeneidad de hábitat y disponibilidad de nutrientes resulta en valores máximos de diversidad de especies y rasgos biológicos, (d) por último, la abundancia y riqueza de los tres grupos biológicos estudiados, zooplancton, fitoplancton y macroinvertebrados, es máxima a niveles de conectividad hídrica crecientes (inundados durante 5, 30 and 40 días al año respectivamente).

En el Ebro Medio, la progresiva regulación de caudales ha alterado la relación entre el río y su llanura de inundación. Como consecuencia, la posibilidad de que se creen nuevos humedales de forma natural es cada vez menor, mientras que los que quedan pierden biodiversidad y funcionalidad debido a la falta de conectividad hídrica. Por tanto, el objetivo de futuros planes de restauración en el Ebro Medio debe incluir el re- establecimiento de un amplio rango de humedales de acuerdo con el potencial sostenible de la llanura. Con este objetivo, diversas actuaciones pueden ser llevadas a cabo, como la modificación de las estructuras de regulación, la creación de humedales artificiales, la re-naturalización de áreas abandonadas o la mejora de la calidad del agua a escala de cuenca.

Resum (Català)

Aproximadament el 90% de les planes d'inundació de grans rius europeus i nord-americans s'han vist fortament modificades a causa d'haver estat regulades, ocupades i contaminades en les últimes dècades, amb importants conseqüències sobre l'estructura i funcionalitat de l'ecosistema fluvial. La resposta d'aquest ecosistema a aquests canvis és complexa, i el seu estudi resulta imprescindible per a una implantació efectiva dels plans de gestió que puguin contrarestar l'impacte negatiu de les activitats antròpiques. Aquesta tesi aporta una base de coneixement per a la restauració d'ecosistemes fluvials, ja que analitza la resposta de les comunitats aquàtiques, especialment macroinvertebrats, als canvis de les condicions hídriques, factors ambientals i pressió antròpica en un gradient lateral de connectivitat hídrica, que es dóna de manera natural, a través de les planes d'inundació d'un riu. La tesi s'emmarca en l'àmbit d'un riu mediterrani, agafant l'Ebre (NE Espanya) com a exemple.

Els resultats obtinguts mostren com la connectivitat hídrica és el factor principal que determina l'estructura d'hàbitats i comunitats aquàtiques en l'Ebre mitjà. Aquesta connectivitat està relacionada amb un ampli ventall de processos que afecten directament o indirecta la presència d'organismes aquàtics, des de l'organització dels hàbitats al transport de nutrients. Així, la connectivitat hídrica és la responsable de com a mínim un terç de la variabilitat observada en la composició d'espècies i els trets biològics de la comunitat de macroinvertebrats, i de més de la meitat de la variabilitat observada en l'abundància i riquesa de macroinvertebrats, zooplàncton i fitoplàncton. Altres factors importants i imprescindibles per a comprendre l'adaptació de les comunitats aquàtiques a la heterogeneïtat natural de les planes d'inundació inclouen variables físiques i químiques, com la terbolesa, salinitat i l'estat tròfic. Per altra banda, el present estudi ha permès identificar tot un seguit de variables relacionades amb les fluctuacions hídriques i que resulten determinants per a les comunitats aquàtiques. Per exemple, el tipus de macroinvertebrats que es troben en cada massa d'aigua ve en gran part determinat per la duració de la connexió que s'estableix entre cada massa d'aigua present en la plana d'inundació i el curs principal del riu , mentre que organismes amb cicles vitals més curts, com el zooplàncton i fitoplàncton, responen millor a les variacions del nivell d'aigua.

La combinació de connectivitat hídrica i característiques físiques i químiques conformen un marc espaciotemporal per al desenvolupament de les comunitats aquàtiques, permetent realitzar prediccions respecte a les característiques taxonòmiques i funcionals de les comunitats aquàtiques. D'acord amb aquest marc ecològic desenvolupat per l'Ebre mitjà, (a) les comunitats aquàtiques en ambients freqüentment inundats, com el riu i braços secundaris estan dominades per larves d'insectes i oligoquets els quals mostren una gran habilitat per a resistir (p.e., mida petita, protecció dels ous) i recuperar-se (p.e. cicle vital curt, reproducció asexual) després d'una pertorbació, (b) per contra, les masses d'aigua que rarament s'inunden estan dominades per crustacis i gasteròpodes que mostren una gran capacitat d'interacció amb altres organismes (p.e. mida gran, reproducció sexual) i que poden utilitzar eficientment els recursos (p.e. elevada diversitat de formes de locomoció i alimentació), (c) en condicions intermèdies de connectivitat hídrica, la combinació d'heterogeneïtat d'hàbitat i disponibilitat de nutrients es tradueix en valors màxims de diversitat d'espècies i trets biològics, (d) l'abundància i riquesa de tres grups biològics com el zooplàncton, fitoplàncton i macroinvertebrats és màxima en nivells de connectivitat hídrica creixents (inundats durant 5, 30 and 40 dies per any respectivament).

En l'Ebre mitjà, la progressiva regulació del cabal ha alterat la relació natural entre el riu i la seva plana d'inundació. Com a conseqüència, la possibilitat de que es creïn noves zones humides de forma natural és cada vegada menor, mentre que les que queden perden biodiversitat i funcionalitat degut a la manca de connectivitat hídrica. Per tant, els objectius de futurs plans de restauració en l'Ebre mitjà han d'incloure el restabliment d'un ampli rang de zones humides d'acord amb el potencial de la plana d'inundació per al seu desenvolupament sostenible. En aquest sentit diverses actuacions es poden dur a terme, com per exemple la modificació de les estructures de regulació del cabal, la creació d'aiguamolls artificials, la restauració d'àrees abandonats, o la millora de la qualitat de l'aigua a escala de conca.

General Introduction

General Introduction

According to Tockner and Stanford (2002), up to 90% of the floodplains in Europe and North America are occupied by agricultural activities, with serious consequences in floodplain functionality. Moreover, by the 2025 time horizon, the future increase of human population will lead to further degradation of riparian areas, intensification of river regulation, an increase in the discharge of pollutants, and further proliferation of invasive species (Tockner and Stanford 2002). Collectively, as floodplain areas become extensively regulated, occupied, and polluted, habitats with low connectivity and high confinement become increasingly vulnerable to the accumulation of pollutants and nutrients generated by human activities. For this reason, it is very relevant to explore the interaction of those factors with the processes that support ecosystem structure and functionality, in order to develop management plans to counteract effectively the influence of negative human impacts (Poff *et al.* 1997, Jansson *et al.* 2000).

In the last years, river restoration has become a major issue and the number of projects and amount of money spent in such restoration projects continuously increases (Jansson *et al.* 2007). However, current restoration projects are often based on trial and error practices (Downs and Kondolf 2002), whereas systematic approaches driven by clearly defined goals and procedures that would ensure the effective use of resources and increase the probability of restoration success, are rare (Schiemer 1999, Buijse *et al.* 2005). Consequently, to be effective, restoration efforts should be based on a solid understanding of biological patterns, as well as functionality and its response to main ecosystem drivers at various spatial and temporal scales (Amoros and Roux 1988, Henry and Amoros 1995, Buijse *et al.* 2002).

Main drivers of floodplain ecosystems

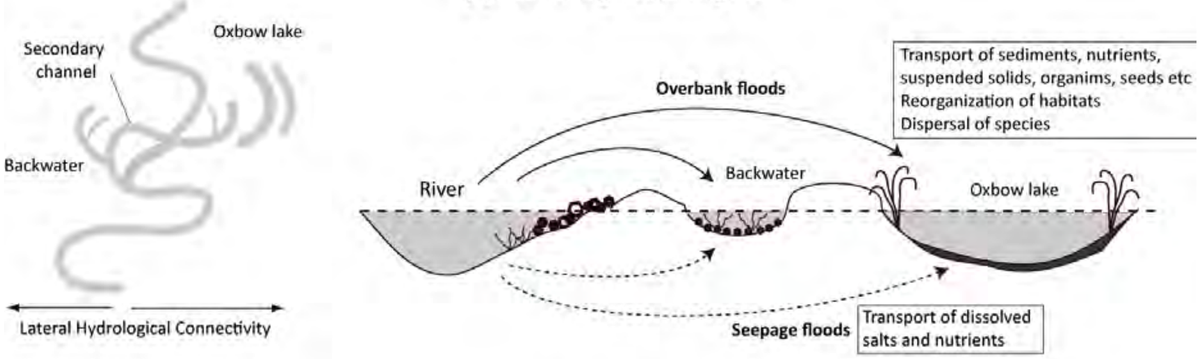
Large rivers form in their floodplains a complex mosaic of interconnected aquatic and terrestrial patches showing different environmental and hydrological features depending on their geo-morphological origin, location within the floodplain and human pressure (Ward and Stanford 1995). Such variety of patches is the base of the extraordinary habitat and biological diversity that characterizes large river-floodplains (Ward *et al.* 1999), which provides a broad range of ecological services ranging from reducing the effect of global changes, to ameliorating the negative impacts of floods, or the self-purification of water (Wantzen *et al.* 2008).

The pulsing of the river discharge generates hydrological gradients through the floodplain, and it is recognized as the main habitat and aquatic community driver in floodplain ecosystems (Junk 1989, Tockner *et al.* 1999a, Amoros and Bornette 2002). Nevertheless, other important drivers of floodplain ecology exist, including environmental factors (e.g., geo-morphological processes, self-spatial organization, bio-geochemical processes and plant dynamics) and human pressure, which in turn, are closely related to the hydrological connection between floodplain habitats and the main river channel (Ward *et al.* 1998).

Hydrological connectivity— Floodplain wetlands (e.g., backwaters, oxbow lakes, temporary ponds) are lentic habitats arranged in a lateral hydrological gradient that become temporarily lotic with river pulses (Fig. 1A) (Amoros and Roux 1988). Although the hydrological regime determines the timing, frequency, intensity and duration of the pulses, several factors beyond the pulsing of the river discharge can influence floodplain hydrological connectivity, including the distance from the river to the wetlands, the presence of natural or artificial barriers to the flow and the morphology of the upstream and downstream wetland borders (Amoros and Bornette 2002). Groundwater seepage, which also makes significant contributions to wetland water levels, is limited by subsurface geological materials and sediment accumulation. The combination of overbank and seepage floods creates a high spatio-temporal heterogeneity and is an important factor contributing to the dispersal of sediments, nutrients, seeds and organisms between inter-connected habitats, thus affecting the productivity and biodiversity of the whole river-floodplain ecosystem (Amoros and Bornette 2002, Wantzen *et al.* 2008).

Physic-chemical gradients— Environmental features (such as the geo-morphological characteristics of the floodplain, the spatial organization of habitats, the characteristics of the substrate, vegetation cover, water physic-chemical characteristics or trophic conditions) have been often identified as important habitat and aquatic community drivers (Wellborn *et al.* 1996, Feld and Hering 2007). In frequently flooded wetlands, the effect of hydrological connectivity on their physic-chemical features depends on the balance between sediment scouring, turbidity, turbulence and nutrient inputs, often resulting in increased suspended sediments and inorganic nutrients (Fig. 1A) (Heiler *et al.* 1995). In contrast, dissolved salts and organic nutrients introduced by groundwater seepage and runoff, or generated by autogenic processes, accumulate in wetlands that are rarely flooded (Heiler *et al.* 1995, Tockner *et al.* 1999a), eventually leading to salinization and eutrophication. Such differences in physic-chemistry between flooded and confined wetlands should be reflected in aquatic communities. For instance, salts and nutrients are directly related with the availability of food and habitat resources for aquatic organisms (e.g., Dolédec *et al.* 2006, Ortiz and Puig 2007, Heino 2008), whereas intermediate levels of salts and nutrients have been related with high overall abundance and diversity of aquatic organisms (e.g., Piscart *et al.* 2006).

(A) Free flowing meandering river



(B) Embanked river

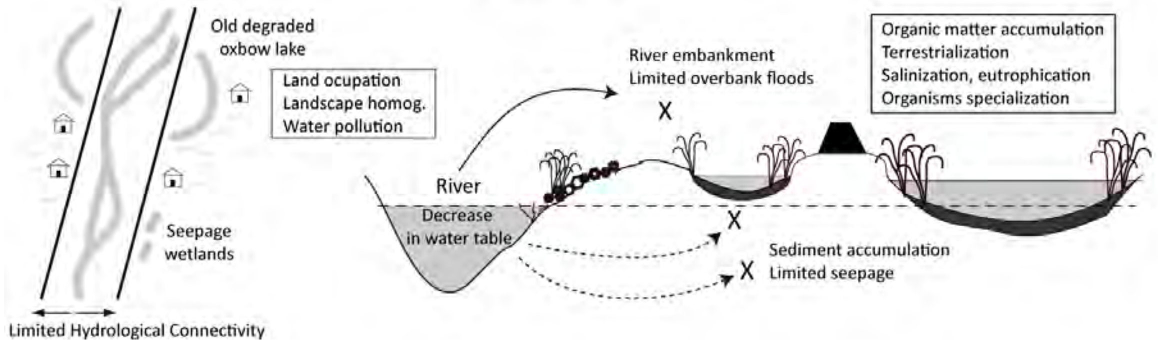


Figure 1 Schematic view of (A) a free-flowing meandering river and (B) a regulated river. The lateral hydrological connectivity between the main channel and floodplain wetlands, such as backwaters and oxbow lakes, is temporarily established during floods. On the left, general view of the floodplain landscape; on the right, transversal view of floodplain processes.

Human pressure— In recent decades, river discharge has been greatly modified by river regulation, which entails a progressive homogenization of the floodplain habitat (Fig. 1B) (Sparks *et al.* 1990, Dynesius and Nilsson 1994, Ward and Stanford 1995). Water retention and diversion, and levee construction changed drastically the hydrological conditions throughout the river ecosystem and caused a decrease of the groundwater level (Petts *et al.* 1989). At habitat scale, the consequences include a lack of rejuvenation in aquatic communities and the accumulation of sediments and organic matter, thereby leading to the salinization and eutrophication of waters (Ward and Stanford 1995). At landscape scale, there are fewer possibilities for new wetlands creation, and those remaining are endangered by high terrestrialization rates (Ward and Stanford 1995). Other important human impacts on stream ecosystems include land occupation and water pollution (Tockner and Stanford 2002). Certainly, the fertile soils and gentle slopes provided by river floodplains combined with the proximity of water, available for consumption, waste spill or transport, make floodplains suitable for intensive agricultural, industrial and urban activities (Jansson *et al.* 2007). Consequences of human pressure on river-floodplain ecosystems range from changes in the taxonomic (e.g., Brabec *et al.* 2004, Woodcock and Huryn 2007) and trait composition (e.g., Dolédec *et al.* 1999, Charvet *et al.*

2000) of aquatic communities enhancing pollution-tolerant species, to the strong reduction of their overall biodiversity and functionality (Erwin 2009).

The dynamics governing the interplay between hydrological, environmental and human gradients across the floodplain is difficult to measure directly (Galat *et al.* 1998, Tockner *et al.* 2000). Nevertheless, it is well-known that present aquatic communities, which exist because of their organisms adaptation to spatial and temporal variability, reflect the historical disturbance pattern of a given site (Batzer and Wissinger 1996). For that reason, changes in the composition and attributes of aquatic assemblages have been widely investigated in an effort to describe hydrological patterns in river-floodplains, as well as to plan and monitor restoration activities (Schiemer 1999, Reckendorfer *et al.* 2006). Among freshwater organisms, invertebrates are particularly useful because of their multiple forms, behaviour and habitat use, allowing them to adapt to environmental gradients (Rosenberg and Resh 1993). Moreover, macroinvertebrates are especially telling regarding ecosystem functionality, biodiversity and environmental alterations (Gasith and Resh 1999). For these reasons, bio-monitoring tools for assessing river alteration in large European and North-American Rivers have frequently focused on macroinvertebrate characteristics (e.g., Rosenberg and Resh 1993, Dolédec *et al.* 1999, Gayraud *et al.* 2003, Statzner *et al.* 2005, Dolédec and Statzner 2007).

Aquatic community patterns across hydrological and environmental gradients

The interplay of hydrological connectivity, environmental features and human pressure provides the potential spatio-temporal habitat available for a species where biological processes operate (Townsend and Hilldrew 1994, Wellborn *et al.* 1996). Within this framework, several hypotheses have been developed in the last decades in order to predict changes in aquatic communities, particularly macroinvertebrates, within the river-floodplain habitat. For instance, the River Habitat Templet (RHT, Townsend and Hilldrew 1994) provides a framework where predictions about the traits of the species more likely to occur under particular spatio-temporal conditions could be made. Thereby, frequent and intensive floods should enhance generalist species with the ability to resist unfavorable conditions (e.g., small body-shape, protection of eggs) or to recover faster when environmental conditions are less limiting (e.g., short life-span, asexual reproduction). In contrast, stability is expected to favor organisms showing the ability to interact and compete with other organisms (e.g., big size, sexual reproduction) and to use efficiently habitat and resources (e.g., several locomotion and feeding strategies) (Townsend and Hilldrew 1994, Townsend *et al.* 1997a). However, despite the fact that these species traits were significantly related to habitat utilization, the overall results of previous investigations showed little agreement with the RHT predictions (Dolédec and Statzner 1994, Resh *et al.* 1994). Such mismatch was related to the high spatio-temporal heterogeneity of the floodplain ecosystems and the correlation between traits (Resh *et al.* 1994).

Regarding aquatic communities diversity, the Intermediate Disturbance Hypothesis (IDH, Connell 1978) predicts overall biodiversity to peak at intermediate levels of hydrological disturbance, which allow both generalist and specialist species to coexist (Fig. 2). While some studies supported the IDH in floodplain habitats (e.g., Ward 1998, Tockner *et al.* 1999b, Amoros and Bornette 2002, Ward *et al.* 2002), others failed, probably because of the complex interaction of hydrological, environmental and biological processes (e.g., Dolédec and Statzner 1994, Resh *et al.* 1994). Moreover, different groups of aquatic organisms responded to hydrological and environmental changes in different ways (Tockner *et al.* 1999b) (Fig. 2). For instance, while fish (Ward *et al.* 1999) and zooplankton (Frisch *et al.* 2005) richness has been reported to be favored by high connectivity, macroinvertebrate species attained their maximum richness in intermediately connected sites (Tockner *et al.* 1999b), amphibians and macrophytes were enhanced by stability in disconnected areas (Tockner *et al.* 1999b), and no significant trends were identified for floodplain vegetation (Pautou and Arens 1994). In a broader sense, it is not clear which are the main drivers of aquatic biodiversity in floodplain habitats, and how aquatic biodiversity is impacted by disruption in natural gradients due to human alteration.

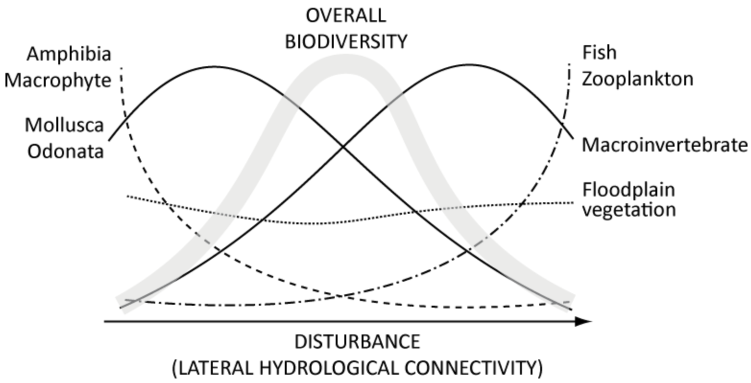


Figure 2 Theoretical distributions of the diversity of different aquatic organisms and overall biodiversity across the lateral hydrological connectivity. Extracted and modified from Connell (1978) and Tockner *et al.* (1999b).

Current situation

The effects of river discharge pulses have been studied in many rivers (Junk and Wantzen 2004). However, despite the key role of the river-floodplain linkages on the structure and functionality of the whole system, there are few studies focusing on floodplain hydrological gradients (Tockner *et al.* 1999a). Moreover, most of the information focuses on a few rivers, such as the Rhône, Danube, Rhine and Elbe (Table 1). All of them are floodplains in temperate climates, so information about Mediterranean, arid and tropical systems is even scarcer.

Table 1 Principal studies analyzing the effect of the river discharge on aquatic invertebrates inhabiting wetlands aligned along the lateral hydrological connectivity.

Stream	Climate	Subject	Reference
Rhône	Temperate	Macroinvertebrate composition (sub-group)	Castella 1984
Rhône	Temperate	Macroinvertebrate composition	Amoros and Roux 1988
Rhône and Ain	Temperate	Macroinvertebrate composition (sub-group)	Castella 1991
Rhône	Temperate	Oligochaeta functionality	Juget and Lafont 1994
Rhône	Temperate	Trichoptera functionality	Tachet *et al.* 1994
Rhône	Temperate	Aquatic insects functionality	Usseglio-Polatera 1994
Rhône	Temperate	Plecoptera and Ephemeroptera functionality	Usseglio-Polatera and Tachet 1994
Rhône	Temperate	Macroinvertebrate functionality	Paillex *et al.* 2007
Upper Rhine	Temperate	Gastropods composition	Obrdlik and Fuchs, 1991
Lower Rhine and Meuse	Temperate	Macroinvertebrate composition	Van den Brink and Van der Velde 1994
Salzach	Temperate	Macroinvertebrate composition	Foeckler *et al.* 1994
Danube	Temperate	Macroinvertebrate richness	Tockner *et al.* 1999b
Rhône, Danube and Rhine	Temperate	Macroinvertebrate biodiversity	Ward *et al.* 1999
Tagliamento	Temperate	Macroinvertebrate composition	Arscott *et al.* 2005
Elbe	Temperate	Molluscs composition and functionality	Foeckler *et al.* 2006
Cooper Creek	Arid	Macroinvertebrate composition	Sheldon *et al.* 2002
Ovens	Arid	Macroinvertebrate composition	Quinn *et al.* 2000
Murray, Darling, Cooper and Diamantina	Arid	Macroinvertebrate composition	Sheldon *et al.* 2006
Flinders	Arid	Macroinvertebrate biodiversity	Leigh and Sheldon 2009
Gregory	Tropical	Macroinvertebrate biodiversity	Leigh and Sheldon 2009
Caloosahatchee	Tropical	Macroinvertebrate functionality	Merrit *et al.* 2002
Parana	Tropical	Macroinvertebrate composition	Zilli *et al.* 2008
Parana	Tropical	Macroinvertebrate composition	Marchese and de Drago 1992

Climatic differences between Mediterranean, temperate, arid and tropical systems (as shown in Figure 3) may influence hydrological patterns and thus, the response of aquatic communities. Mediterranean streams are characterized by high spatial and temporal heterogeneity that offers habitats for numerous rare or endemic species (Myers *et al.* 2000). These characteristics make Mediterranean streams an ecosystem of great ecological value, despite being confined to small regions of the world (between 1-4% of the continental area, mostly located in the Mediterranean basin) (Gasith and Resh 1999). Moreover, the impact of river regulation is higher in Mediterranean than in other climatic areas because of its natural variability, higher vulnerability, and poorer recovery capacity (Gasith and Resh 1999, Bonada *et al.* 2007). Consequently, there is an unmet need to integrate the study of rivers and their floodplains, particularly in Mediterranean regions. Such lack of information may be related to the lack of naturally fluctuating systems, since most of the European and North American floodplains have been regulated for centuries (Wantzen *et al.* 2008). This lack of knowledge is most remarkable as floodplain occupation and water diversion increases continuously, and all climate-change models consistently predict increases in extreme climatic events, and reductions in water availability (Wantzen *et al.* 2008).

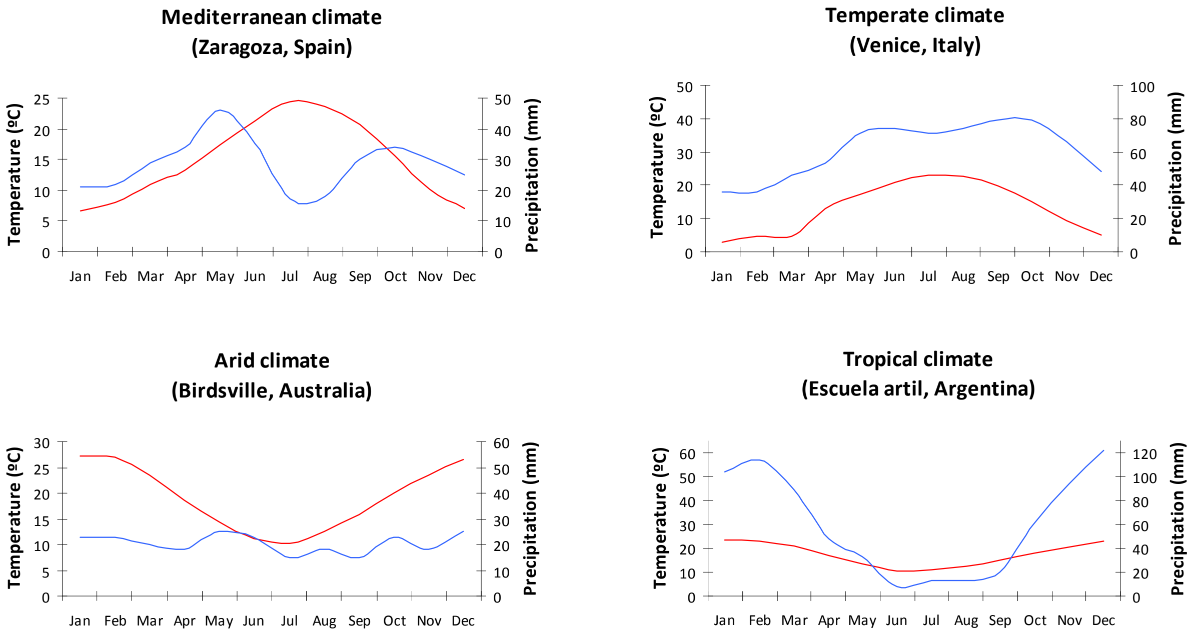


Figure 3 Rainfall (blue) and temperature (red) diagrams corresponding to different climatic regions. The present study focused in a Mediterranean river. Data extracted from www.globalbioclimatics.org.

Concluding, although the investigation of hydrological gradients has become a major issue in river ecology, several questions remain to be answered. For instance, given the actual scenario of increased water demand and climate change, which would be the habitats and aquatic communities' response to future changes in the hydrological regime? Are environmental gradients more or less important than hydrological connectivity in structuring habitats and aquatic communities, or do they have interacting effects on floodplain ecosystems? To what extent do human activities disrupt natural hydrological and environmental gradients? How does such disruption affect the distribution of species? Will a Mediterranean system be more or less drastically affected by human alteration and climate change than other climatic systems? The answers to these questions are not simple and require an integrating approach, but it is clear that the river-floodplain linkages and their disruption by human activities need further research, especially for rivers undergoing deep transformations due to climate change, river regulation or ecological restoration.

Objectives

Taking into account (a) the important ecological, social and economic value of the river-floodplain ecosystem, (b) the key role of the interaction between hydrological connectivity, environmental gradients and human pressure in determining the structure, biodiversity and functionality of the ecosystem, (c) the strong reduction in natural gradients caused by flow diversion and regulation in the last decades, (d) the special vulnerability of Mediterranean rivers to reductions in flow quantity, quality and variability, and (e) the role of aquatic communities, specially macroinvertebrates, in the river-floodplain environment, which makes them reliable indicators of higher-scale processes, the main goal of the present thesis is:

> To provide a solid background for river-floodplain restoration through the response of aquatic communities, especially macroinvertebrates, to changes in hydrological connectivity, environmental factors and human pressure across the lateral hydrological connectivity in a large Mediterranean river.

This main goal will be accomplished by means of five specific objectives that cover both the taxonomic and functional aspects of aquatic communities, in an attempt to improve basic knowledge, but also to provide guidelines to management actors. These objectives are structured as follows (Fig. 4):

Taxonomic approach:

> (i) To identify main hydrological and environmental factors determining the taxonomic composition and diversity of aquatic communities across the lateral hydrological connectivity.
>
> (ii) To model and predict the response of floodplain aquatic assemblages to the lateral hydrological connectivity.

Functional approach:

> (iii) To identify main hydrological and environmental factors determining the functional composition and diversity of aquatic communities across the lateral hydrological connectivity.
>
> (iv) To test the response of the macroinvertebrate functional composition and biodiversity to flooding and confinement patterns.

Management guidelines:

(v) To use the available information (key factors influencing the composition, diversity and functionality of aquatic communities) to provide guidelines for the conservation and ecological restoration of the study area.

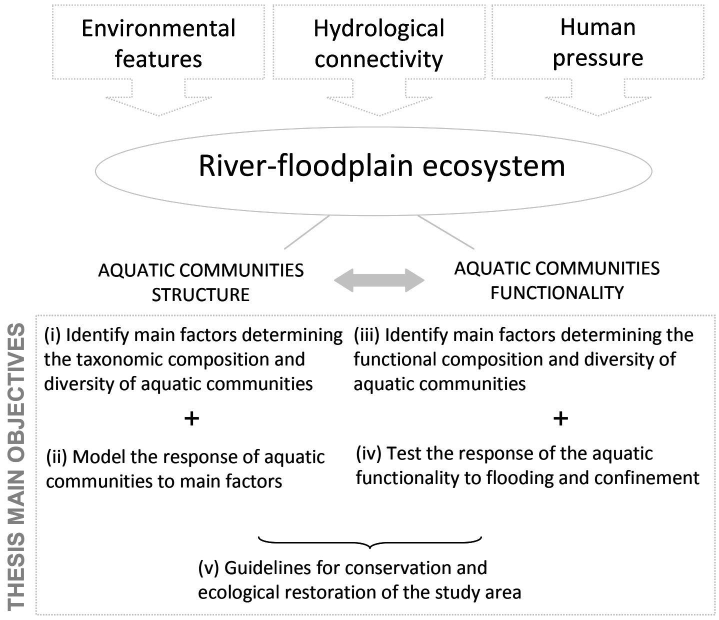


Figure 4 Schematic view of main objectives (i-v) accounted for in this thesis.

Study Approach

Following the scheme provided in Figure 4, **Chapter 1** aimed to investigate the key hydrological and environmental factors determining the presence and abundance of macroinvertebrates in the Middle Ebro floodplain. To this end, wetlands were identified by their hydrological connectivity with the main river channel and it was hypothesized that the macroinvertebrate assemblages of those wetlands, quantified through diversity and abundance measurements, would show significant differences. Invertebrate attributes (such as abundance, richness, diversity and evenness) at wetlands arranged in a lateral hydrological connectivity were further investigated to test the Intermediate Disturbance Hypothesis (Connell 1928). **Chapter 1** has been published with modifications in *Aquatic Sciences* (70: 248-258).

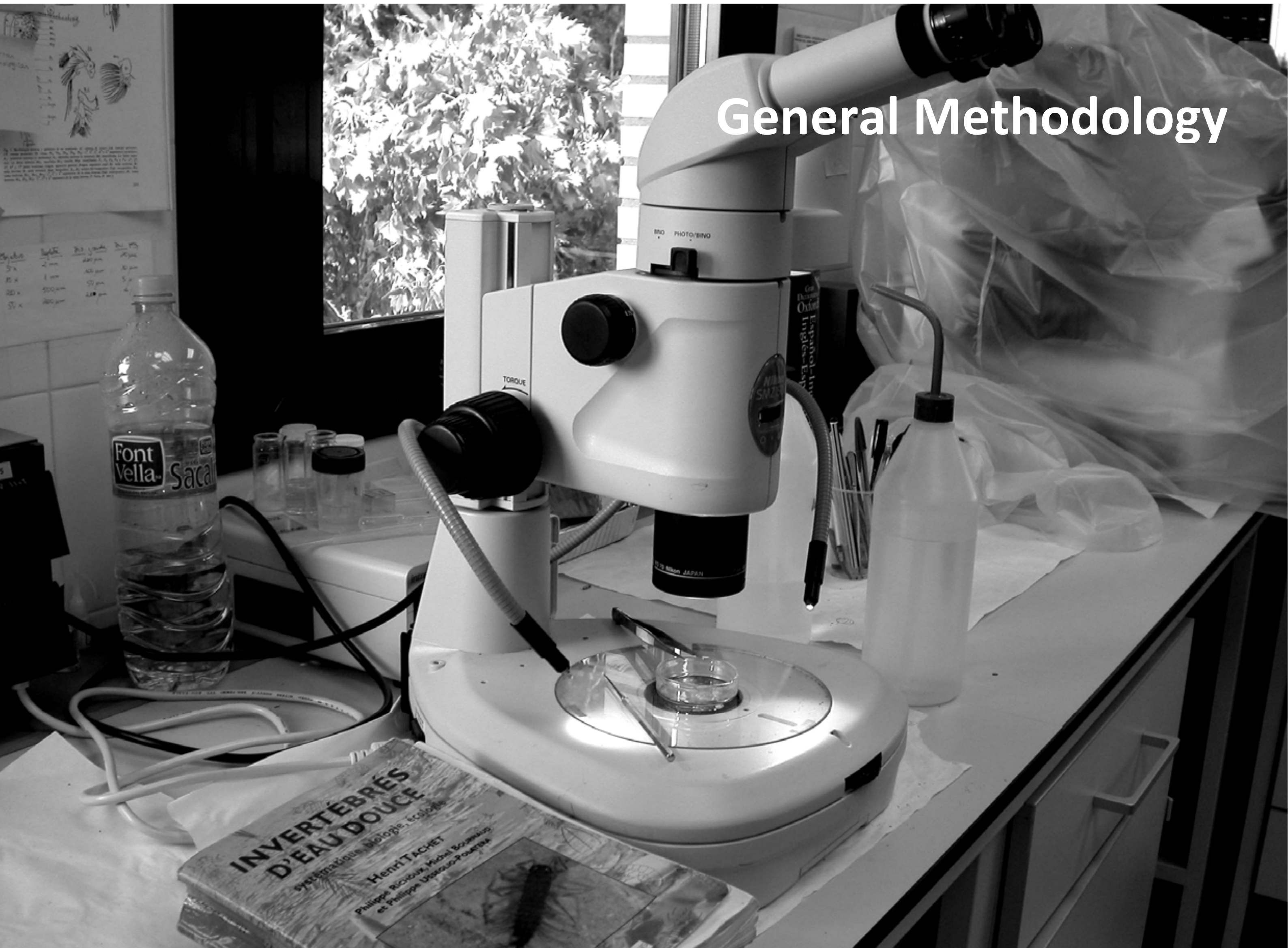
Once the key role of hydrological connectivity was proved in general terms for macroinvertebrate assemblages in **Chapter 1**, we tested the ability of six different surrogates of hydrological connectivity (e.g., flood duration, magnitude and frequency) to model patterns of biological features (such as richness, abundance and composition), considering several aquatic groups. The simultaneous study of macroinvertebrates, zooplankton and phytoplankton provided an indication of the effect of hydrological connectivity on the aquatic food web. Through this analysis, we aimed to select those hydrological surrogates that best accounted for variability in aquatic assemblages features and that will be useful for planning and executing restoration in the study area. This objective was accounted for in **Chapter 2**, which has been accepted for publication in *Marine and Freshwater Research*.

After the taxonomic approach to the Middle Ebro floodplain was fully accomplished, we focused on the relationships between environmental variables and aquatic community functionality using invertebrate biological traits (e.g., respiration, reproduction or feeding behaviour) in **Chapter 3**. Our first objective was to identify significant differences in trait composition, trait richness and trait diversity between wetlands aligned along the lateral hydrological connectivity. As the taxonomic and functional compositions of invertebrates are usually considered to be shaped by the same environmental gradients, we hypothesized that key gradients extracted in **Chapter 1** will exert a significant influence in the relative abundance of functional traits. This chapter has been published in *Fundamental and Applied Limnology* (173: 281-292).

Similarly, in **Chapter 4** we aimed to investigate the relative importance of environmental features related to flooding and confinement to explain the macroinvertebrate trait composition in the Ebro floodplain wetlands. To this end, we used data from a higher geographical scale sampling (Middle Ebro sector scale) that allowed us to test the simultaneous relationships of environmental variables, species abundance and species traits through an improved statistical procedure, the fourth-corner analysis (Dray and Legendre 2008). Differences in trait composition and biodiversity between flooded and confined sites were also assessed. Chapter 4 has been accepted for publication in *Journal of Limnology*.

Finally, the **General Discussion** section tried to gather the solid understanding provided in **Chapters 1-4** to (i) summarize findings about aquatic community patterns across hydrological and environmental gradients in the Middle Ebro floodplain, (ii) discuss these findings in the framework of other temperate, tropical and arid streams, (iii) examine how this information can be used to provide guidance for restoration projects in the area of study.

General Methodology



General Methodology

Study Area

The area of study was located in the middle sector of the Ebro River, in NE Spain. With a catchment area of 8,093 km^2 and a length of 910 km, the Ebro is a large Mediterranean river and the longest river in Spain. In its middle sector, near the city of Zaragoza, the Ebro is a meandering river (sinuosity = 1.39, slope = 0.05%) with an average 5-km width floodplain. At Zaragoza gauging station, average discharge is 230 m^{3}s^{-1} (Spanish Water Authority, URL: http//: www.chebro.es).

The Ebro River environmental problems are similar to those faced by other rivers in Europe and North America. In the past 150 years, humans have canalized, polluted, and impounded this river for agricultural, urban, and industrial purposes. In the last century, and more intensively since the 60's, the floodplain of the Ebro River have been extensively affected by changes in land-use and construction of structures to control flood (Cabezas 2008). The combination of disturbance pulses and natural river succession in the Ebro river-floodplain has historically created numerous wetlands that have varying degrees of hydrological connection as, for example, secondary channels, backwaters, oxbow lakes, and temporary pools (Ollero 1996). However, the lack of intensive, erosive floods in the last decades has reduced the probability of creation of new wetlands. Consequently, most of the floodplain area (more than 60%) is used for agriculture whereas wetlands cover only 3.6% of it (Cabezas *et al.* 2008).

Sampling scales

We performed several samplings during the years 2006 and 2007, with different frequencies (bi-monthly and annual) and spatial scales (stretch and sector), depending on the particular objectives of the study.

Stretch-scale— The sampling performed in 2006 every two months (6 times) included four natural oxbow lakes (NOL), two constructed oxbow lakes (COL) and the main river channel (RS), all of them located within Natural Reserve Areas (Table 2 and Fig. 5). The stretch-scale sampling covered a 13-km of the river catchment. The study sites were located within natural reserve areas and represented the widest range of hydrological connectivity within the river floodplain system, from permanently flooded (RS) to rarely flooded (NOL) or seepage-flooded (COL) wetlands. This sampling was intended to provide intensive and detailed multivariate information on a set of 6 wetlands and the Ebro River. Sampling included water chemistry and macroinvertebrate communities, and resulting information was used in Chapters 1-3. In addition, variations in the water-column and plankton (zooplankton and

phytoplankton) communities were monitored in three of these wetlands (NOL1-3) to further assess the effect of hydrological connectivity on the aquatic food web.

Table 2 General characteristics of wetlands included in the stretch-scale. Depth is measured at the deepest part of the wetlands in summer.

Wetland	**Depth (m)**	**Age (yr)**[A]	**Area (ha)**[B]	**River Dist (m)**[C]	**Urban Dist (m)**[C]	**Substrate**	**Vegetation cover**	**Hydrological connectivity**
RS2	-	-	125.5	0	1520	Gravel	Emergent/ Submergent	Permanently
NOL1	0.8	42	70.31	1077	4141	Silt	Emergent	Frequently
NOL2	1.73	61	35.45	868	2418	Silt / Clay	Emergent	Intermediate
NOL3	2.42	61	10.33	833	3066	Silt	Emergent	Rarely
NOL4	0-1.10	61	4.82	269	1704	Silt / Clay	Emergent/ Herbaceous	Rarely
COL1	0.5	2	3.07	518	3133	Gravel	Emergent/ Submergent	Seepage
COL2	0.5	1	2.08	528	2817	Gravel	None	Seepage

[A] Age is the time since the cut-off of oxbow lakes, or construction, depending on origin.
[B] Area was measured using summer aerial photographs.
[C] River and urban distances are the minimum distance from each wetland to the river channel or the nearest urban area.

The Ebro River was representative of the highest level of hydrological connectivity and was the main source of water, organisms, and energy through flow and flood pulses to riparian wetlands. One sampling station was established in the river channel at the centre of the study area. Although the point selected in the river channel was located 500 m downstream from the Zaragoza wastewater treatment plant, the environmental conditions of this site are not significantly different from other sites in the river channel (personal observation), which are characterized by high nutrient loads. As reported by Torrecilla *et al* (2005), agricultural, industrial and urban development in the Ebro basin has a significant impact on its water quality, particularly on the nitrate concentration that is high in its middle sector (between 10 and 30 mg L^{-1}). In addition, other authors have observed the effect of waste plants on the benthic community to diminish 300 m downstream (Ortiz and Puig 2005).

NOLs showed similar habitat characteristics in terms of morphology, vegetation and substrate texture. They were small (35–70 ha) and shallow (0.5–2 m of depth) former river meanders, 42 to 61 years old, that were separated from the river channel by a strip of riparian vegetation and a thick layer of sediment limiting groundwater seepage. Substrate size was similar in NOLs and COLs, with 70-90% of fine and sand materials (particles < 500 µm), which contrasted with the high percentage of coarse materials (90% particles > 500 µm) in RS (Fig. 6). NOLs were surrounded by emergent vegetation, mostly *Phragmites australis* and *Typha latifolia*, but lacked submergent vegetation. They were flooded at different flow limits (from 400 to 1200 m^3s^{-1}) depending on distance to the river and channel embankment, and were thus arranged in a gradient of lateral hydrological connectivity.

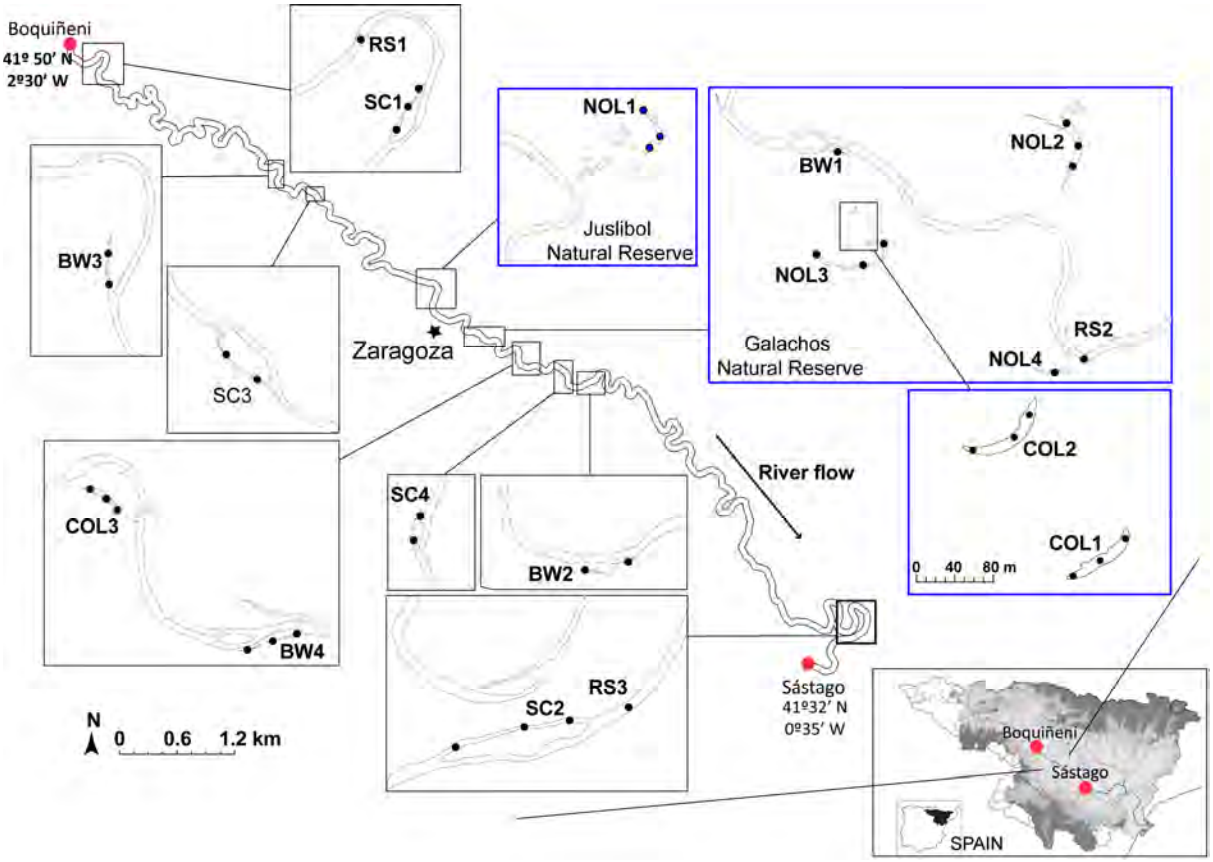


Figure 5 Localization of the study area in the middle sector of the Ebro River. RS= River sites, SC= secondary channels, BW= backwaters, NOL= natural oxbow lakes, COL= constructed oxbow lakes. Scale bar is for every enlarged area, except for COL1 and COL2 that present a different scale. Black dots are sampling points were water and macroinvertebrate samples were taken. Every wetland was included in the Middle Ebro-scale sampling. Wetlands in the blue enlarged areas were also included in the stretch-scale samplings. Distance from Boquiñeni to Sástago is 100 km approximately.

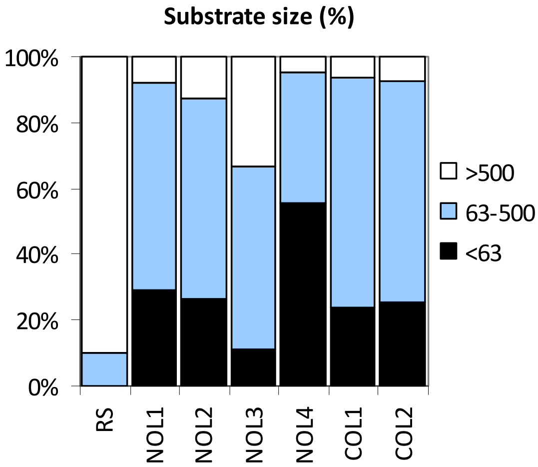


Figure 6 Substrate size as percentage of particles showing different sizes (in μm) in wetlands included in the stretch-scale sampling.

Both COL1 and COL2, which were constructed close to NOL3 to mitigate wetland loss, have gravel substrata that allow water seepage from the river and hill-slope aquifers. However, their relatively high elevation does not allow for a surface connection with the river at flows below 2500 m^3s^{-1}, which has probably contributed to the lack of fish in these systems. Aquatic vegetation has rapidly colonized COL1 (*Typha latifolia* and submerged macrophytes), but COL2 remains un-vegetated.

Middle Ebro sector scale— A higher scale sampling was performed with an annual frequency in 2006 and 2007 (twice), including 17 wetlands that covered a 100-km long stretch of the river catchment. This sampling was intended to complement the stretch-scale sampling by increasing three fold the number of sampling points, thus increasing the accounted spatial variability and leading to reliable results. At this larger geographical scale, variables related to water chemistry and macroinvertebrate communities were used. Sampling sites included three stations located at the river channel (RS), four side channels (SC), four backwaters (BW), four natural oxbow lakes (NOL) and three constructed oxbow lakes (COL) (Fig. 5, Table 3).

Table 3 General characteristics of wetlands monitored at the Middle Ebro sector-scale.

Wetland	**X**^{A}	**Y**^{A}	**Vegetation**	**Substrate**	**Area (ha)**^{B}	**Human alteration**	**River Dist**^{C}	**Urban Dist**^{C}
RS 1	646357	4635935	Emergent/ Submergent	Gravel / Silt	-	Agriculture / Natural	0	1573
RS 2	647186	463572	Emergent/ Submergent	Gravel / Silt	-	Agriculture	0	2054
RS 3	686790	4605968	Emergent/ Submergent	Gravel / Silt	-	Agriculture	0	1520
SC 1	646869	4635188	Emergent/ Submergent	Gravel	2.22	Agriculture	138	1535
SC 2	722320	4577914	Emergent/ Submergent	Gravel	7.5	Agriculture/ Industrial	215	250
SC 3	668249	4621761	Submergent	Gravel	2.09	Agriculture	162	1708
SC 4	684208	4608171	Herbaceous	Sand	0.23	Agriculture / Natural/ Urban	75	2528
BW 1	697289	4603333	Emergent	Silt / Clay	1.74	Agriculture	64	4506
BW 2	694396	4602628	Herbaceous	Gravel	2.1	Agriculture / Impoundment	103	4967
BW 3	664268	4623910	Herbaceous	Gravel	2.45	Industrial/ Impoundment	215	1216
BW 4	689689	4604661	-	Gravel	0.94	Agriculture / Industrial	32	1075
NOL 1	672608	4619264	Emergent	Silt	70.31	Agriculture/ Natural	923	3836
NOL 2	686752	4608237	Emergent	Silt / Clay	35.45	Agriculture/ Natural	868	2418
NOL 3	684438	4606963	Emergent	Silt	10.33	Agriculture/ Natural	833	3066
NOL 4	686499	4605879	Emergent	Silt	4.82	Agriculture/ Urban	269	1704

COL 1	684595	4607221	Emergent/ Submergent	Gravel/ Silt	0.25	Agriculture/ Natural	546	3086
COL 2	684412	4607559	-	Gravel	0.58	Agriculture/ Natural	528	2817
COL 3	688219	4606339	Emergent	Gravel/ Silt	4.86	Agriculture/ Fish	344	1038

[A] UTM geographical coordinates are referred to the North hemisphere, zone 30.
[B] Area was measured using summer aerial photographs.
[C] River and urban distances are the minimum distance (m) from each wetland to the river channel or the nearest urban area.

Similar to the sampling performed at the stretch-scale, RS provided the reference for river conditions in terms of hydrological connectivity, water chemistry or invertebrate composition. The number of sampling points in the river channel increased from one at the stretch-scale, to three sampling points (Fig. 5) at the Middle Ebro sector-scale.

Although SCs showed lower water current than the main river channel, they were permanently connected at their upstream and downstream ends with the river channel. SCs were surrounded by riparian vegetation, and showed emergent and submerged vegetation. Substrata in SCs were mainly composed of gravels and sand.

BWs were surrounded by emergent vegetation and presented a fine sediment substratum due to high sediment inputs from the river. BWs were connected to the main channel at their downstream end, but were reconnected at their upstream end at intermediate river discharge (200-400 m^3s^{-1}). Some of them (e.g., BW2) were close to industrial parks or waste water treatment plants (e.g., BW5).

The natural oxbow lakes sampled at the Middle Ebro sector-scale were the same as those sampled in the stretch-scale.
Three COLs were monitored at the Middle Ebro sector-scale. Two of them (COL1 and COL2) are the same described in the stretch-scale. COL3 was an older constructed lake for gravel extraction and actually used for sport (fishing) purposes. Consistently with COL1 and COL2, COL3 had gravel substrata and was surrounded by emergent vegetation. In contrast, COL3 was bigger, deeper and was flooded with higher frequency than COL1 and COL2.

Hydrological setting

Even though the Ebro floodplain has been hydrologically disconnected from the main river, the flow in the middle section is relatively variable, and conserves its dynamism. In fact, overbank floods (i.e. those exceeding the floodplain level, established at 600 $m^3\ s^{-1}$) occur on average 33 days per year, while river discharge is at its lowest (< 30 m^3s^{-1}) during summer, which occurs on average 47 days every year (mean values from 1980-2006, discharge data from the Spanish Water Authority, URL: http//: www.chebro.es).

Between 12 and 16 March 2006, an extraordinary flood (1,586 m^3s^{-1}, 2-yr return period, Fig. 7) raised the floodplain water level to a point where all of the floodplain wetlands, except COLs, were connected to the river. Excluding this flood event, the river flow remained unusually low in 2006 (average discharge in 2006= 143 m^3s^{-1}; average discharge from 1912 to 2003= 235 m^3s^{-1}, Fig. 7). Another extraordinary flood took place between 25 March and 16 April 2007 (2,282 m^3s^{-1}, 10-yr return period, Fig. 7). Photographs from this flood episode can be consulted in Appendix 5. In contrast to the dry 2006, 2007 can be considered closer to the average conditions (average discharge= 228 m^3s^{-1}).

By the time we performed the sampling, the wetlands could be considered to be fully recovered from flood events. Nevertheless, the wetland connectivity caused by the 2006 and 2007 floods provided an opportunity to assess wetland response to surface connection gradients across the floodplain.

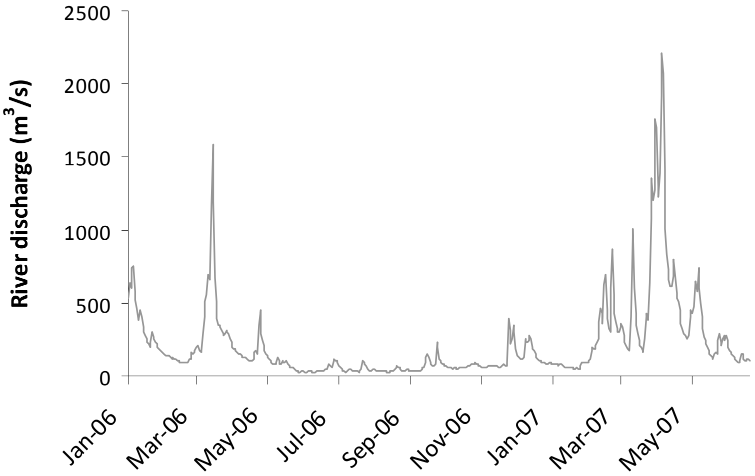


Figure 7 Hydrograph of the Ebro River at Zaragoza gauging station between January 2006 and July 2007.

Beyond river flow discharge, water-level in each NOL was recorded continuously using pressure-based meters with 1-cm resolution (DI-241 Diver, Van Essen Instruments®). One data logger was located at the center of each NOL, so that the same value of water-level was assigned to the three sampling points located within each NOL. Several surrogates of hydrological connectivity were calculated from water-level data provided by data-loggers and aerial photographs, which are summarized in Table 4.

Table 4 Hydrological features used to study the effect of hydrological connectivity on floodplain aquatic communities.

Hydrological feature*	Abbreviation	Units	Description
River Distance	RD	m	Minimum distance from each sampling point to the river channel
Flood Limit	FL	m^3s^{-1}	River flow limit to overbank flood
Flood Magnitude	FM	%	Increase in water-level relative to the minimum water-level in the study period
Flood Duration	FD	days	Number of days/yr that the river flow rose above the flood limit
Flood Frequency	FF	nº	Number of events/yr that the river flow rose above the flood limit
Water level variability 7 days	WLV 7	-	Coefficient of variation of the water-level over a period of 7 days before each sampling date in each wetland
Water level variability 30 days	WLV 30	-	As above but in a 30 days period
Water level variability 180 days	WLV 180	-	As above but in a 180 days period
Water level variability 365 days	WLV 365	-	As above but in a 365 days period

* To note that hydrological features were only available for NOL1-3 monitored at the stretch-scale, and not for other wetlands included in the stretch- or Middle Ebro sector-scales.

Sampling procedure

Two to three sampling points depending on the area (see sampling points in Fig. 6) were located in each wetland, and one sampling point in the river channel, for environmental and biological monitoring. Sampling was similar at both scales, thus only the number of wetlands monitored and the sampling frequency varied. An example of sampling procedure (samples and variables measured) in a floodplain wetland is shown in Figure 8. The general procedure is described below and the specific methodologies (e.g., supplementary variables, statistical analyses) are described in each chapter.

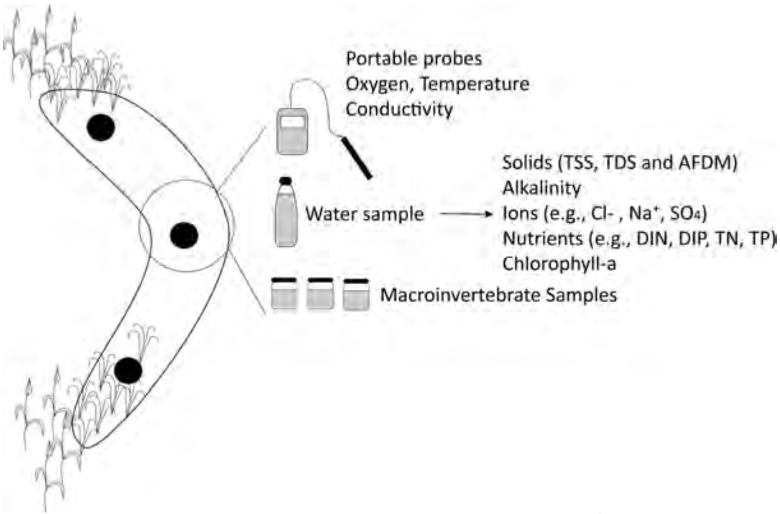


Figure 8 Example of sampling procedure in a floodplain wetland. In each sampling point (black dots) physic-chemical variables were measured *in situ*, a water sample was taken to the laboratory for chemical analyses, and three macroinvertebrate samples were collected at different habitats.

Environmental monitoring

At each sampling point, dissolved oxygen, temperature, conductivity and pH were measured *in situ* using portable probes previously calibrated (WTW® Multiline P4). Two-litre water samples were collected directly into acid-washed polycarbonate bottles at a depth of 20 cm and placed on ice. On the same day, samples were filtered through Whatman® GF/F glass fibre filters (pre-combusted at 450ºC for 4 h) to determine the amount of suspended, dissolved and ash-free solids (APHA 1989). Alkalinity was measured using potentiometric automatic titration with 0.04 N H_2SO_4 (APHA 1989). Ion chromatography was used to determine dissolved nutrient and anion concentrations (Br^-, Cl^-, SO_4^{2-}, F^-, NO_3^-, NO_2^-, PO_4^{3-}, K^+, Mg^{2+}, Na^+ and NH_4^+) and a continuous flow analyzer (FLOWSYS-SYSTEA®) was used to determine total nitrogen (TN) and total reactive phosphorous (TP) (APHA 1989). Organic fractions (DON and DOP) were calculated as the difference between totals (TN, TP) and inorganic fractions (DIN, DIP) respectively. Finally, phytoplankton photosynthetic pigments were analyzed using the Spectrophotometric Method (APHA 1989). Results from these analyses can be consulted in Appendix 1.

Biological sampling and identification

A sweep net (45 x 45 cm frame net, 500 µm sieve, 1 min. sampling) was used to collect invertebrates (catches per unit effort, CPUE) at various microhabitats within each wetland. The microhabitats included emergent vegetation (e.g., *Phragmites australis* and *Typha latifolia*, which were present in dense stands along the margins of most floodplain wetlands), leaf packs (coarse organic matter), riverbank areas that were free of vegetation and had a silt or gravel substratum, and stagnant waters. Samples were preserved in 5% formalin and then washed through nested sieves (2500 µm, 1000 µm, and 500 µm). Samples were hand-sorted under a stereoscopic microscope, and organisms were identified to the lowest taxonomic level, usually genus. The results from different habitats sampled in each sampling point were pooled together. A taxa list of benthic macroinvertebrates found in different habitats (e.g., RS, SC, BW) of the Ebro ecosystem can be found in Appendix 2.

Statistical analyses

Given the high amount of data gathered, special attention has been devoted to the statistical analysis of the hydrological, environmental and biological data sets. To extract significant patterns, traditional statistics (such as multivariate analysis or regression models) and modern applied statistics (such as complex regression models or the fourth-corner method) have been used in combination. To that end, several statistical software programs were used, such as SPSS 15.0, CANOCO 4.5 and R 2.5.1. The latter, R 2.5.1, has been increasingly used because of its wide flexibility and applications, and the statistical functions used in each chapter are listed in Appendix 4. Because of their complexity, the specific statistical analyses will be described in each chapter.

Results

Chapter 1

Main environmental factors determining the taxonomic composition and diversity of aquatic communities across the lateral hydrological connectivity [1]

Abstract

Flood and flow pulses are primary factors that regulate macroinvertebrate community structure in river-floodplain ecosystems. In order to assess the impacts of these important hydrological events in a regulated Mediterranean river, bimonthly water and macroinvertebrate samples were collected in 2006 in the Ebro River and six floodplain wetlands. We found significant differences among the river sites (permanently flooded), two constructed wetlands (seepage flooded) and three natural oxbow lakes (surface flooded at 400, 800 and 1200 m^3s^{-1}). River sites were dominated by aquatic worms (e.g., Naididae) and showed a high degree of eutrophication. Constructed wetlands provided new habitat for predatory insects (e.g., *Coenagrion scitulum* and *Trithemis annulata*) that benefited from the absence of fish. Natural oxbow lakes were dominated by crustaceans (e.g., *Atyaephyra desmaresti*, *Procambarus clarkii*) and were highly overlapped in Correspondence Analysis. Canonical Correspondence Analysis, coupled with variance partitioning, showed that hydrological connectivity accounted for 28% of the variability in the invertebrate community, followed by physic-chemical (10%) and trophic (7%) factors. Differences in frequency and duration of flood pulses in natural oxbow lakes were not enough to generate distinctive macroinvertebrate assemblages in the different wetlands. In contrast, richness and total abundance increased with hydrological connectivity, while diversity showed a rather unimodal distribution, consistent with the Intermediate Disturbance Hypothesis. Generalized Additive Models indicated that among the measured environmental variables, nitrate strongly affected the abundance, diversity and evenness of aquatic communities. Our data highlight that increasing the diversity of waterbody types in degraded floodplains enhances biodiversity and aids in the functional recovery of the river landscape.

Introduction

The structure and function of river-floodplain ecosystems are directly, and indirectly, affected by river discharge fluctuations (*sensu* “flood” and “flow pulses”, Junk 1989, Tockner *et al.* 2000). Hydrological connectivity involves patterns and processes across the river-floodplain system, such as transport of suspended or dissolved elements and organisms, reorganization of habitats, productivity and biodiversity of aquatic and terrestrial communities (Amoros and Roux 1988, Ward and Stanford 1995, Tockner *et al.* 1999a). Several factors beyond river flow fluctuations can influence floodplain

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[1] This chapter has been published with modifications as follows: Gallardo, B., M. García, A. Cabezas, E. González, M. González, C. Ciancarelli and F. A. Comín. 2008. Macroinvertebrate patterns along environmental gradients and hydrological connectivity within a regulated river-floodplain. *Aquatic Sciences* 70: 248-258

hydrological connectivity. The distance from the river to the wetlands is directly related to overbank flood frequency wherever there are no natural or artificial barriers to water flow. The morphology of the upstream and downstream wetland borders is related to the magnitude and duration of surface connectivity with the main channel (Amoros and Bornette 2002). Groundwater seepage, which also makes significant contributions to wetland water levels, is limited by subsurface geological materials and sediment accumulation. The combination of these features results in a shifting mosaic of aquatic and terrestrial patches that provides highly diverse aquatic habitats, communities and processes within the river-floodplain system (Ward 1998).

Given that higher habitat diversity will promote increased biological diversity, aquatic assemblages (from phytoplankton to fish) provide an adequate measure of habitat structure and function at different spatial and temporal scales, and are often used to assess hydrological patterns (Rosenberg and Resh 1993). Among freshwater organisms, invertebrates are particularly useful because of their short life cycles and multiple adaptation strategies (e.g., body form, cycles per year, life span, feeding habitats) to environmental gradients (Gasith and Resh 1999). Since many benthic invertebrates exhibit a sessile life style, their presence can be particularly telling about habitat quality. Moreover, specific groups of macroinvertebrates show overlapping optima of abundance and diversity along both spatial and environmental gradients, although little is known about them (e.g., Tockner *et al.* 1999b, Arscott *et al.* 2005).

In this study, we investigated the relationships between physical, chemical and biological characteristics of floodplain wetlands in the middle sector of the Ebro River (NE Spain). We identified wetlands by their hydrological connectivity with the main river channel and hypothesized that the macroinvertebrate assemblages of those wetlands, quantified through diversity and abundance measurements, would demonstrate significant differences. We also identified multiple environmental gradients that affect aquatic communities and, by partitioning the variability of those gradients, we were able to test their separate importance in explaining invertebrate community variability. We further investigated differences in invertebrate attributes (e.g., abundance, richness, diversity and evenness) between wetlands arranged along a lateral hydrological connectivity and hypothesized that according to the Intermediate Disturbance Hypothesis (Connell 1978), diversity will increase at intermediate levels of hydrological disturbance. In addition, we modeled the response of invertebrate attributes against a gradient of nitrate concentration, which reflects agricultural land-use in the Ebro catchment, an activity that occupies more than the 60% of the catchment (Ollero 1996). Finally, we addressed the effect of wetland creation at the scale of the floodplain and discuss the implication of our results for river-floodplain conservation and restoration.

Material and Methods

Selected sites for this Chapter included four natural oxbow lakes (NOLs), two constructed oxbow lakes (COLs) and the main river channel (RS) corresponding to the stretch scale-sampling (Fig. 5). Selected study sites were representative of the range of hydrological connectivity in the river floodplain and were located within Natural Reserve Areas, which prevents them from being impacted by uncontrolled human activities. However, they may be affected by non-point sources on nutrients because of the extensive agricultural land-use in the Ebro River catchment (more than 60% of the floodplain area) (Ollero 1996). Water and macroinvertebrate samples were taken every two months in 2006 from various locations at the seven study sites according to the methods described in General Methodology (stretch-scale sampling).

Because of the lack of previous studies in this section of the Ebro River, all available environmental information was considered in Chapter 1. Dissolved oxygen, temperature and pH were measured in situ using portable probes, whereas dissolved, suspended and ash-free dissolved solids were calculated in the laboratory from water samples (APHA 1989). Dissolved anions (Mg^{2+}, SO_4^{2-}, Br^-, Cl^-, K^-, Na^+, Ca^+, HCO_3^-), nutrients (NO_3^- , NO_2^-, DON, PO_4^{3+}, DOP) and photosynthetic pigments (Chl-a) were calculated following standard methods (APHA 1989).

Aquatic organisms were sampled in triplicate in each sampling point to be sure that every habitat was covered. Macroinvertebrates were identified to genus level and were grouped into broad taxonomic groups (e.g., Oligochaeta, Macrocrustacea, Diptera) when necessary. To quantify the ecological characteristics of the macroinvertebrate community, we used a set of attributes that included total abundance (N), genera richness (R), the Shannon-Wiener Diversity Index (H) and evenness (J) calculated according to Pielou (1969).

Hydrological connectivity definition

In order to classify wetlands by their hydrological connectivity, we considered several factors at landscape and wetland scales. River regulation and lack of flow fluctuations negatively affects all of the study sites, but especially those located farther from the river channel, where connections are established only during extraordinary floods. As a consequence, the intervals between “reconnection” events have been growing longer in the last century (e.g., 3,000 m^3s^{-1} return period has shifted from 10 to 60 years in the last decades). River to wetlands distance is usually related to the frequency of hydrological connectivity (Robinson *et al.* 2004); however, the height of the river embankment is also important. For instance, levee construction at the downstream end of some natural oxbow lakes (e.g., NOL2 and NOL4) limits surface connectivity and requires higher river flows to top the high banks. Morphology of upstream and downstream wetland borders impacts both the flow and turnover of water during flood pulses. In addition, dense stands of emergent vegetation are a natural biological filter of water entering natural oxbow lakes, causing a drop in water velocity and concurrent settling of suspended material. On the other hand, sediment accumulation has been enhanced by the lack of intensive, erosive floods during the last few decades, thus reducing groundwater seepage in natural oxbow lakes. Consequently, when and how long a floodplain wetland is connected to the main river channel varies with river flow and other wetland characteristics.

To assess macroinvertebrate assemblages along a gradient of hydrological connectivity, we constructed a categorical variable called Type, which had five categories (Type 1, 2, 3, 4 and 5), based on the characteristics of each wetland as substrate size and flood frequency (Table 5). Lack of spatial replication of hydrological connectivity types was compensated with a high temporal replication. Flood limit in RS was taken to be 200 m^3s^{-1}, at which the lentic habitats sampled in the river shore became lotic. NOLs were sequentially surface-flooded at 400, 800 and 1200 m^3s^{-1}, depending on the particular wetland. COLs were considered to be seepage flooded all the year because they never dried-off. Flood limits were based on a detailed Digital Elevation Model (DEM), coupled with field measurements. Connectivity, therefore, was not treated as a continuous variable, but rather a typology that involved multiple patterns and processes throughout the river floodplain system. In addition, river water level fluctuation (WLV-river) was taken into account as the coefficient of variation of the river flow 30 days before each sampling date.

Statistical analyses

First, to assess general patterns in the distribution of environmental variables and broad taxonomic groups (11 invertebrate groups), differences between wetlands and seasons were assessed with non-parametric analysis of variance (Kruskal-Wallis test, $p \leq 0.05$).

After that, patterns in the species composition (48 invertebrate genera) was further analysed by means of indirect ordination methods, such as Correspondence Analysis (CA) (ter Braak and Šmilauer 2002). To that end, we reduced the effect of absolute values by square-root transforming ($\sqrt{X+1}$) the abundances of the invertebrate densities, and downweighted rare taxa following ter Braak and Šmilauer (2002).

Table 5 Hydrological connectivity definitions for wetlands in the Ebro River. "Type" corresponds to connectivity categories. FL= river flow limit to overbank flood; FF= flood frequency, number of perturbation events when the river flow exceeds the flood limit in 2006; FD= flood duration, total number of days flooded in 2006.

Type	Depth (m)[A]	Age (yr)[B]	Area (ha)[C]	FL (m^3s^{-1})	FF (nº)	FD (days)	Hydrological Connectivity Definition
1	-	-	125.5	200	30	71	**Surface flooded.** Gravel substrata. Includes RS.
2	0.8	42	70.31	400	3	25	**High surface-flooded.** A thick layer of sediment reduces groundwater connectivity. Includes NOL1.
3	1.73	61	35.45	800	2	7	**Moderate surface-flooded**. A thick layer of sediment limits groundwater connectivity. Levee construction limits overbank floods. Includes NOL2.
4	0-2.42	61	4.82-10.33	1200	1	3	**Low surface-flooded.** A thick layer of sediment limits groundwater connectivity. Includes NOL3 and 4
5	0.5	0.5-1	0.25-0.58	2500	0	0	**Seepage-flooded**. Gravel substratum that allows groundwater seepage from both river and hillslope aquifers. Topography doesn't allow surface connection, even at extraordinary floods. Includes COL1 and 2.

[A] Depth is at the deepest part of the wetlands in summer.
[B] Age is the time since the cut-off of oxbow lakes, or construction, depending on origin.
[C] Area was measured using summer aerial photographs.

To identify the primary environmental gradients affecting macroinvertebrate assemblages, we used Canonical Correspondence Analysis (CCA, ter Braak and Šmilauer 2002) between 23 environmental variables and the abundance of 11 invertebrate groups. Environmental variables were previously log (X+1) transformed, with the exception of pH and hydrological connectivity (introduced as five dummy variables: Type 1, 2, 3, 4 and 5). Because environmental gradients had not previously been evaluated in the study area, we ran a manual forward selection, which included variables that had a conditional effect significant at the 10% level ($p \leq 0.1$, Magnan 1994). In addition, the inflation factor of selected variables was check to be less than 10, ensuring a minimum redundancy among the retained variables (ter Braak and Šmilauer 2002). *P*-values were calculated using the Monte Carlo Permutation Test (Hope 1968). Once the main environmental variables affecting the aquatic community composition were identified, the model was subjected to variation partitioning in order to quantify the amount of variation uniquely explained by the variables included the model, previously divided into three groups: physic-chemical, trophic and hydrological. The shared variance among the three groups and the variance that remained unexplained were also calculated (Borcard *et al.* 2004). Multivariate analyses were carried out with CANOCO 4.5 (ter Braak and Šmilauer, 2002).

Differences in invertebrate attributes (i.e., abundance, richness, diversity and evenness) between wetlands arranged in a lateral hydrological connectivity were tested (Kruskal Wallis test, $p \leq 0.05$) and graphically expressed in boxplots.

Finally, Generalised Additive Models (GAM, Wood 2006) with a maximum of three degrees of freedom to reduce the complexity of the models (Castella *et al.* 2001), were used to test the relationship between the invertebrate attributes and the nitrate concentration surrogating agriculture land-use (Smith *et al.* 2007). The regression models were run using the statistical software R (version 2.5.1; R Development Core Team, 2007; “mgcv” package). Functions used in this Chapter can be consulted in Appendix 4.

Results

Spatial patterns in environmental conditions and macroinvertebrate assemblages in the Middle Ebro River

Mean 2006 annual values for the measured hydrological, physic-chemical and trophic parameters, and significant differences among hydrological connectivity types in the floodplain of the Ebro River, are shown in Table 6. The wetlands differed mainly in their inorganic ion concentrations (TDS, HCO_3^-, NO_3^-, PO_4^{3-}) and organic (ADFM, DON, DOP, Chl-*a*) elements. COLs and NOL2 had significantly higher dissolved solids and nitrate concentrations but lower organic matter contents than the rest of the wetlands. NOL3 and 4 had significantly higher chlorophyll-*a* concentrations. Finally, NOL1 showed intermediate concentrations of both organic and inorganic elements.

Table 6 Water characteristics of six wetlands and the Ebro River. Data are mean (SD) of bimonthly samples in 2006. TDS = Total dissolved solids, AFDM = ash-free suspended solids, T = temperature, DO = dissolved oxygen, Chl-*a* = Chlorophyll-*a*, DON = dissolved organic nitrogen, DOP = dissolved organic phosphorous.

	RS (n=6)	**NOL1 (n=18)**	**NOL2 (n=18)**	**NOL3 and 4 (n=36)**	**COL1 and 2 (n=36)**
<u>Hydrological variables</u>					
Type	1	2	3	4	5
WLV-river	0.46 (0.25)	0.47 (0.22)	0.55 (0.31)	0.42 (0.20)	0.56 (0.30)
<u>Physic-chemical variables</u>					
TSS (mg L^{-1})	87.6 (81.1)	39.4 (33.8)	34.8 (33.4)	40.7 (62.52)	20.4 (13.7)
TDS (mg L^{-1})*	1002 (304)	1779 (952)	1824 (459)	1982 (731)	3586 (1391)
AFDM (mgL^{-1})*	13.9 (6.1)	8.08 (4.33)	14.2 (31.7)	17.2 (11.5)	5.94 (3.12)
Mg^{2+} (mg L^{-1})*	28.4 (7.3)	41.8 (20.9)	41.9 (4.6)	45.7 (18.2)	87.4 (43.4)
Na^+ (mg L^{-1})*	166.6 (49.0)	285.6 (163.9)	306.6 (40.6)	182.2 (59.3)	391.3 (115.6)
Ca^+ (mg L^{-1})*	152.6 (69.7)	204.1 (70.2)	180.9 (16.0)	2.52 (0.27)	279.1 (179.0)

SO_4^{2-} (mg L^{-1})*	502.4 (322.0)	720.9 (385.2)	534.4 (91.4)	1157 (493)	1841 (900)
Fl^- (mg L^{-1})*	0.20 (0.03)	0.10 (0.00)	0.10 (0.04)	0.25 (0.08)	0.29 (0.08)
Br^- (mg L^{-1})*	0.20 (0.08)	0.20 (0.10)	0.50 (0.09)	0.15 (0.90)	0.45 (0.38)
Cl^- (mg L^{-1})*	330.8 (102.2)	575.5 (392.1)	582.6 (101.7)	356.1 (112.7)	779.0 (249.0)
K^- (mg L^{-1})*	6.20 (2.90)	7.90 (2.00)	4.00 (1.00)	10.0 (2.30)	7.35 (2.91)
HCO_3^- (mg L^{-1})*	164.5 (87.0)	126.7 (68.9)	273.4 (71.4)	132.1 (31.4)	141.0 (56.8)
pH*	8.10 (0.30)	8.00 (0.30)	7.70 (0.20)	7.85 (0.30)	7.91 (0.23)
T (ºC)	14.5 (3.5)	16.4 (7.3)	13.1 (3.30)	15.7 (6.60)	16.93 (5.60)
DO (mg L^{-1})	8.50 (2.70)	7.20 (2.00)	8.60 (1.70)	8.89 (5.21)	9.36 (1.59)
Trophic variables					
Chl-*a* (µg L^{-1})*	21.6 (18.3)	14.4 (17.2)	7.40 (11.2)	36.9 (26.9)	3.56 (3.00)
NO_3^- (mg L^{-1})*	16.0 (4.6)	1.80 (3.10)	42.9 (7.00)	1.20 (3.00)	47.02 (42.1)
NO_2^- (mg L^{-1})*	0.20 (0.10)	0.02 (0.01)	0.10 (0.10)	0.05 (0.12)	0.05 (0.05)
DON (mg L^{-1})*	0.40 (0.50)	0.10 (0.20)	0.02 (0.08)	0.28 (0.28)	0.07 (0.14)
PO_4^{3+} (µg L^{-1})	5.00 (0.00)	5.00 (0.00)	5.00 (0.00)	24.7 (64.5)	9.30 (11.6)
DOP (µg L^{-1})*	12.7 (11.1)	4.40 (4.20)	4.60 (3.80)	28.0 (49.2)	2.50 (3.40)

*= significant differences (nonparametric Kruskal Wallis test, $p \leq 0.05$) among wetlands

Mean annual macroinvertebrate abundance and significant differences among hydrological connectivity Types are shown in Table 7. We recorded 77,200 individual macroinvertebrates belonging to 48 genera and 11 broad taxonomic groups. Crustacea and Oligochaeta were the most common invertebrate groups (35% each, by abundance). Wetlands differed mainly in their abundance of oligochaetes, insects and crustaceans as shown by Kruskal-Wallis test.

Table 7 Invertebrate composition and attributes of six wetlands and the Ebro River. Data are mean (SD) from bimonthly samples in 2006. R = Invertebrate richness, N = Total invertebrate abundance, H = Invertebrate Shannon diversity, J = Invertebrate evenness.

	RS (n=6)	**NOL1 (n=18)**	**NOL2 (n=18)**	**NOL3 and 4 (n=36)**	**COL1 and 2 (n=36)**
Type	1	2	3	4	5
N*	2824 (2573)	643 (477)	123 (163)	279 (305)	1030 (915)
R*	11.2 (2.7)	11.33 (4.45)	8.10 (2.60)	6.12 (2.33)	8.20 (3.50)
H*	0.59 (0.26)	1.11 (0.48)	1.33 (0.29)	0.80 (0.41)	0.98 (0.62)
J*	0.09 (0.05)	0.21 (0.09)	0.37 (0.10)	0.29 (0.24)	0.20 (0.16)
Hirudinea*	3.50 (7.62)	0	0	0	0
Coleoptera	0.08 (0.20)	0.16 (0.31)	0.03 (0.13)	0.13 (0.31)	0.47 (0.83)
Diptera*	124 (179)	67.4 (78.9)	9.90 (12.70)	23.7 (33.4)	289 (333)
Ephemer.*	23.1 (49.0)	24.6 (48.9)	2.77 (7.60)	0.80 (1.59)	74.0 (88.6)
Gastropoda*	13.6 (32.1)	10.5 (26.5)	0.27 (0.46)	0.33 (0.56)	0.13 (0.52)
Heteroptera*	244 (297)	54.1 (186.6)	1.70 (2.71)	0.17 (0.33)	19.3 (46.0)
Macrocrust.*	1.75 (1.80)	1.90 (3.00)	27.5 (64.2)	0.15 (0.38)	0
Microcrust.*	23.2 (36.2)	390 (418)	60.7 (95.4)	215 (278)	82.9 (179.9)
Odonata*	0	3.45 (9.76)	0.07 (0.17)	0.24 (0.56)	292 (603)
Oligochaeta*	2266 (2294)	15.9 (16.8)	9.40 (11.00)	14.0 (36.9)	1.73 (2.60)
Trichoptera*	1.50 (1.61)	4.05 (5.96)	0.73 (1.25)	1.25 (2.70)	0.07 (0.26)

*= significant differences (non-parametric Kruskal Wallis test, $p \leq 0.05$) among wetlands.

The main river channel (RS) was clearly dominated by aquatic worms, though insects were also abundant. COLs showed the lowest abundance of macroinvertebrates and were dominated by insects. The four NOLs were dominated by crustaceans although insects were abundant as well (Fig. 9).

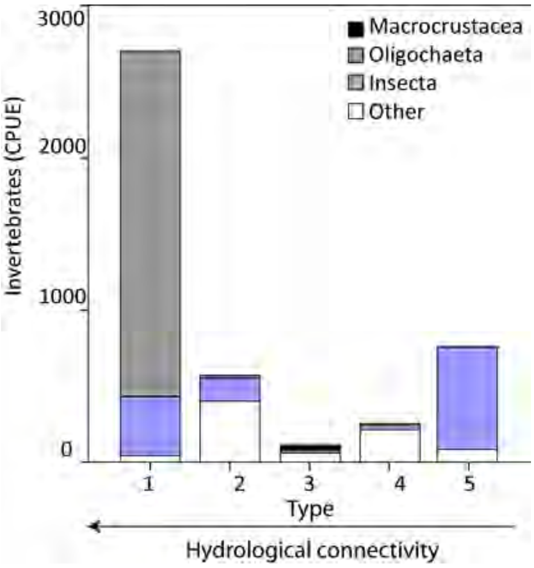

Figure 9 Abundance of main invertebrate groups in six wetlands and the Ebro River arranged in a lateral hydrological connectivity by Type (see hydrological definitions in Table 5). Differences between wetlands are significant (Kruskal-Wallis test, $p<0.001$). CPUE= catches per unit effort.

Results from the Correspondence Analysis (CA) model, performed with the abundance of 46 invertebrate genera, were consistent with patterns in broad taxonomic groups assessed with Kruskal-Wallis test. First and second ordination axes accounted for 28% of the initial variance, and indicated a gradient from COL habitats at the left, to NOL habitats at the right sides of the plot (Fig. 10). RS habitat was related to the abundance of aquatic worms belonging to the Naididae family, though aquatic bugs (e.g., *Micronecta* sp.) and midges (e.g., *Chironomus* sp., *Orthocladius* sp.) were also abundant. COLs showed a relationship with the abundance of Odonata (e.g., *Trithemis annulata, Coenagrion scitulum*), Ephemeroptera (e.g., *Caenis luctuosa, Baetis fuscatus*) and Diptera families (e.g., Chironomidae, Ceratopogonidae). The four NOLs were reported to be related to the abundance of crustaceans. In particular, macrocrustaceans (e.g., *Atyaephyra desmaresti, Echinogammarus* sp*., Procambarus clarkii*) were more abundant in highly flooded wetlands (NOL1) than in NOL2-4 (Fig. 10).

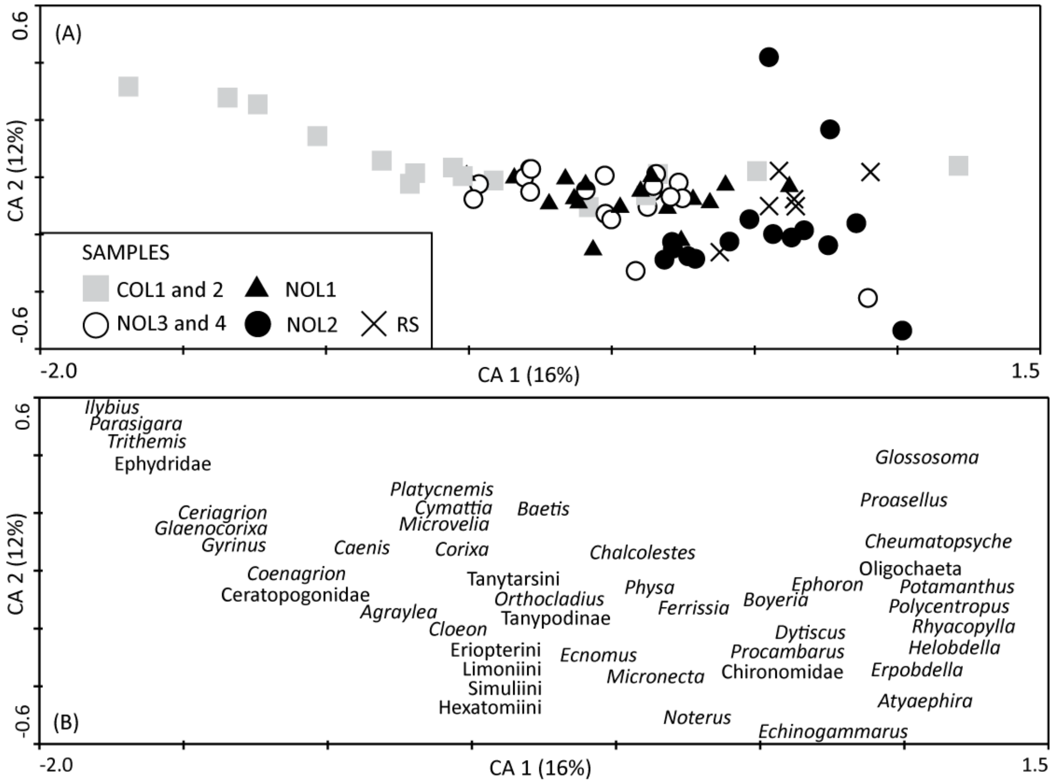

Figure 10 Results of Correspondence Analysis (CA) performed with invertebrate data from the Ebro River and six floodplain wetlands. (A): plot of sample scores. (B): plot of genera scores. Labels are located at the centre of each species score.

Temporal patterns in environmental conditions and macroinvertebrate assemblages in the Middle Ebro River

Only 6 out of 22 environmental variables showed significant differences (Kruskal-Wallis *p*<0.05) across seasons including temperature, alkalinity, dissolved oxygen, bromure, organic phosphorous and nitrogen (Fig. 11).

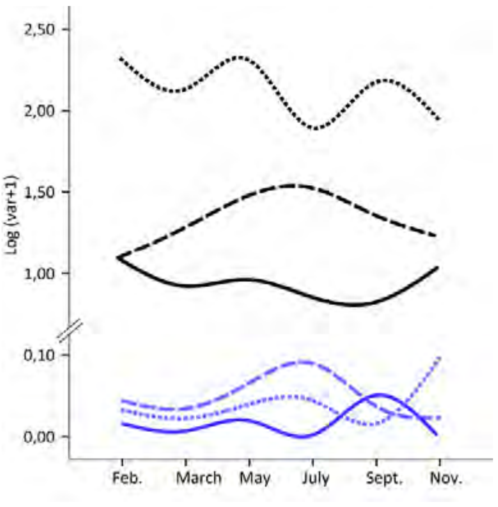

Figure 11 Environmental variables showing significant differences (Kruskal Test, *p*<0.05) between months.

Regarding main invertebrate groups, analysis of variance revealed that microcrustaceans were significantly more abundant in February while Odonata were more abundant in September. Macroinvertebrate richness was significantly highest in May. Detailed information on macroinvertebrate composition and richness fluctuations over time can be consulted in Appendix 3.

Primary gradients affecting aquatic community structure

Of the initial 23 environmental variables included in the Canonical Correspondence Analysis (CCA), 12 were retained as significant contributors (Type, WLV-river, TDS, AFDM, Mg^{2+}, SO_4^{2-}, Cl^-, K^-, DO, Chal-a, NO_3^- and DON). The non-retained variables were redundant or did not increase the significance of the model. The final CCA accounted for 65% of the total variance in invertebrate composition and all canonical axes were significant (Monte Carlo test, $p = 0.002$). In the ordination space of Factors 1 and 2, COLs, NOLs and RS habitats were clearly distinguished by environmental variables and invertebrate composition (Fig. 12). RS appeared at the upper left corner and was mainly related to the nitrate concentration and to the abundance of Oligochaeta and Heteroptera. COLs, located at the upper right side of the plot, showed higher salts concentration (TDS, Cl^-, SO_4^{2-} and Mg^{2+}) and high abundance of insects belonging to Odonata, Ephemeroptera, Diptera and Coleoptera families. Surprisingly, there were no apparent differences among the NOLs despite they differ in their lateral hydrological connectivity. NOLs were located at the lower left side of the plot and reported higher organic nutrient concentration (AFDM), primary productivity (Chl-a), fluctuations in the water level (WLV-river) and potassium (K^-). Regarding their invertebrate assemblages, NOLs were related to the abundance of crustaceans (both Macro and Microcrustaceans) and Trichoptera.

Variance partitioning showed that the hydrological set (including Type and WLV-river) accounted for 28% of the invertebrate variability. Retained physic-chemical variables (DO, K^+, Cl^-, TDS, SO_4^{2-} and Mg^{2+}) explained 10%; while trophic variables (NO_3^-, DON, chlorophyll-*a* and AFDM) explained 7% of the invertebrate variability. Shared variance between the three sets of variables accounted for 20% of the invertebrate variability; 35% remained unexplained (Fig. 12).

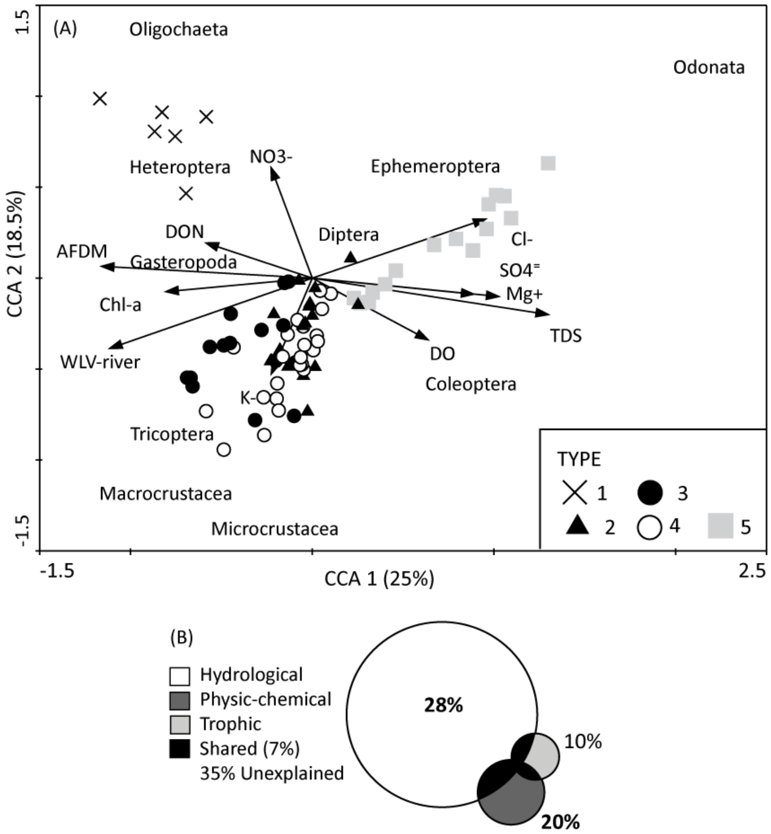

Figure 12 Results of Canonical Correspondence Analysis (CCA) performed with invertebrate and environmental data from the Ebro floodplain using forward selection of variables (p <0.1). (A) Triplot of significant environmental variables, invertebrates groups and sample scores; (B) Results of variation partitioning.

Response of invertebrate attributes to hydrological and agricultural gradients

Analysis of variance (Kruskal-Wallis test) reported that greater hydrological connectivity was associated with greater invertebrate richness and total abundance. Shannon diversity and evenness showed a rather unimodal shape across hydrological types (Fig. 13).

The Generalized Additive Models indicated a contrasting response of macroinvertebrate abundance and richness on the one side; and macroinvertebrate diversity and evenness on the other (Fig. 13). Whereas the former were highest at intermediate nitrate concentrations of 10 mg L^{-1}, the latter were lowest. Furthermore, macroinvertebrate diversity and evenness were highest at high nitrate concentrations, of 40 mg L^{-1} approximately. Conversely, such high nitrate concentration exerted a negative effect on the abundance and richness of macroinvertebrates. Despite the model developed for macroinvertebrate richness was not significant (p = 0.13), it was included for comparison, as its response against nitrate concentration is similar to that of macroinvertebrate abundance.

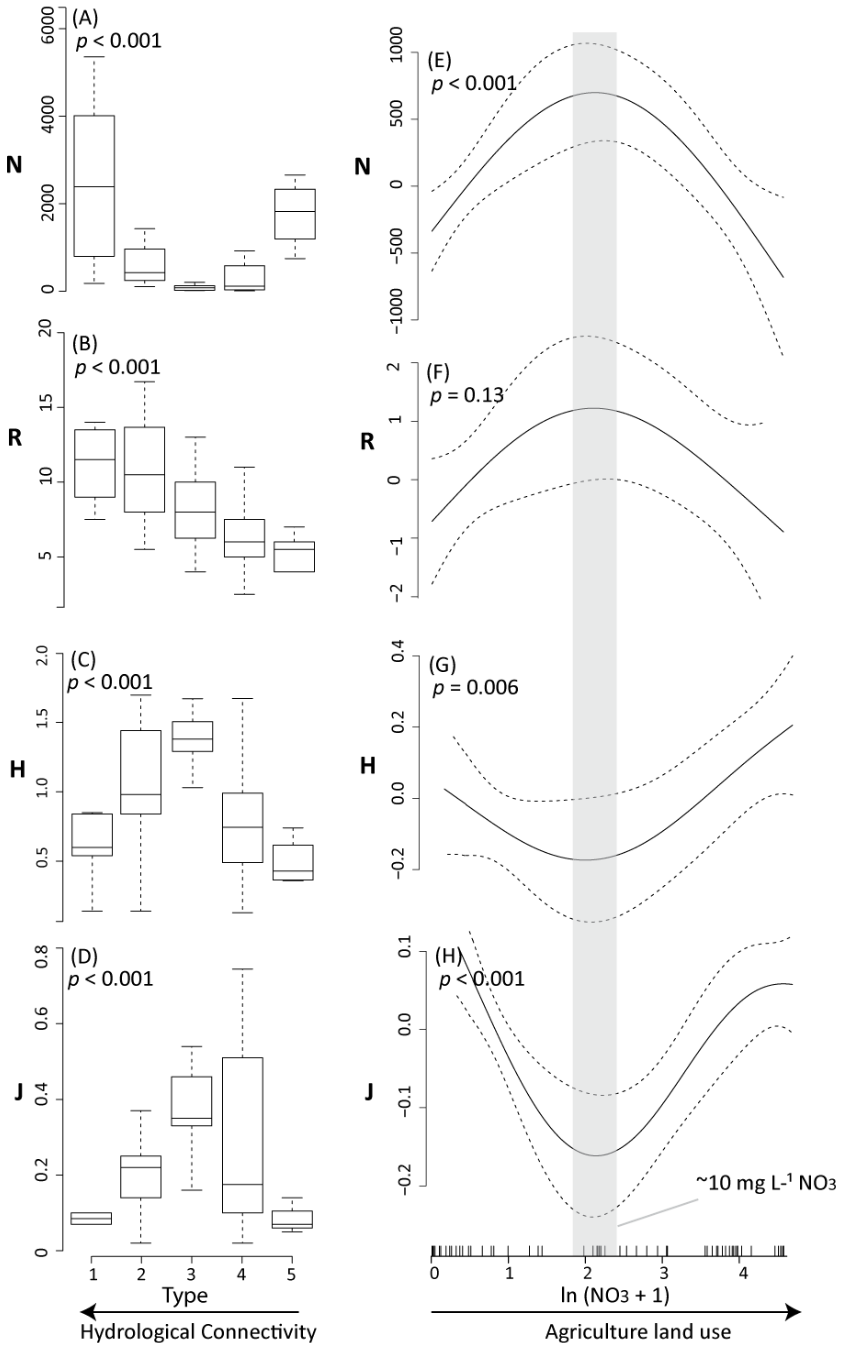

Figure 13 (A-D) Box-plots of macroinvertebrate attributes in six wetlands and the Ebro River arranged by their lateral hydrological connectivity. (E-H) Generalized Additive models performed between macroinvertebrate attributes and nitrate concentration. Dashed lines represent the 95% confidence interval for the model. N= Invertebrate total abundance; R= Invertebrate richness; H= Invertebrate Shannon diversity; J= Invertebrate evenness.

Discussion

Environmental conditions in the Middle Ebro River

Wetlands investigated in this study showed distinctive water chemistry mainly reflected in the concentration of organic and inorganic constituents. In floodplain wetlands, suspended and dissolved solids from allochthonous (overbank flooding or seepage) or autochthonous (internal primary production and proximal riparian ecosystems) sources, accumulate in floodplain wetlands during periods of confinement (Heiler *et al.* 1995, Tockner *et al.* 1999a). Nevertheless, oxbow lakes can become a source of these compounds during large, intensive floods (Junk 1989, Tockner *et al.* 1999a). The frequency of flood pulses increases turbidity and the concentration of inorganic nutrients, whereas the lack of flood pulses increases sedimentation rates and eutrophication in floodplain wetlands (Tockner *et al.* 1999a). Consistently, wetlands that were more frequently connected to the river, as NOL1 and NOL2, showed a high concentration of suspended solids and inorganic nutrients, while wetlands that were confined most of the time, as NOL3 and NOL4 showed a high organic nutrient concentration, which may foster increased primary productivity through stability and nutrient availability. NOL2 was separated from the other NOLs by its higher nitrate concentration, which probably originated from the intensive agricultural development on the surrounding land. In contrast, denitrification in the benthic layers of NOL3 and 4 may be responsible for the very low inorganic nitrogen concentrations in those wetlands. Temporal differences in environmental conditions are related to the natural increase in temperature and concentration of dissolved compounds in confined systems during summer.

Macroinvertebrate assemblages in the Middle Ebro River

In our study of the Ebro floodplain, non-insect taxa, particularly aquatic worms and crustaceans, were numerically dominant components of the wetland macroinvertebrate communities, as in other rivers (e.g., Arscott *et al.* 2005, Whiles and Goldowitz 2005). Analysis of Correspondence showed a gradient from invertebrates with effective dispersal and resistance strategies such as insects, towards non-insect invertebrates that inhabit more stable environments, such as crustaceans, snails and aquatic worms (Fig. 10). Organic matter pollution in the river channel, downstream a waste water treatment plant, enhanced pollution-tolerant species such as aquatic worms and midges. On the other hand, long periods of stability in oxbow lakes have favoured the development of taxa that have relatively long life cycles and less effective colonization strategies, such as crustaceans (Gasith and Resh 1999). Finally, insects that have short life cycles (e.g., chironomids, ceratopogonids and culicids) were abundant in newly-constructed oxbow lakes which can be colonized from the air, and where larger predatory insects benefit from the absence of fish (Mallory *et al.* 1994).

Statistical analyses reflected the relatively small differences in NOLs macroinvertebrate assemblages, even in situations where wetlands differed substantially in environmental conditions (organic and inorganic elements) and their hydrological connection to the main river channel. Initially, based on the hydrological relationships with the river, we identified three types of NOLs (Types 2, 3 and 4), but differences in the lakes were not sufficient to generate measurable differences in invertebrate composition and abundance. Nevertheless, differences in hydrological connectivity may be reflected in macroinvertebrate community attributes not quantified in this study, such as biomass, diversity within taxonomic groups and trait composition (e.g., Heino 2000, Griffith *et al.* 2001).

Primary gradients affecting aquatic community structure

The critical influence of floods and flow pulses on aquatic communities in river-floodplain ecosystems has been evaluated by various researchers (e.g., Boulton *et al.* 1992, Tockner *et al.* 1999b, Sheldon *et al.* 2002, Arscott *et al.* 2005, Whiles and Goldowitz 2005, Reese and Batzer 2007). In the current investigation, we identified several variables that impact wetland hydrological connectivity, such us natural or artificial barriers to river flow, morphological characteristics of upstream and downstream wetland borders, subsurface geological materials and sediment accumulation.

Certainly, as shown by CCA analysis combined with variation partitioning, the most important factor associated with macroinvertebrate assemblages in the Ebro floodplain was hydrological connectivity with the river channel. A major finding was that hydrological connectivity explained a substantial amount of the macroinvertebrate variance, even after the variation shared with other significant variables was removed. Certainly, in this and other studies, the remainder of the variance is generally attributed to environmental factors, such as nutrients and temperature (e.g., Heino 2000, Zimmer *et al.* 2000, Griffith *et al.* 2001). However, the impact of these factors may be somewhat spurious since even temperature and nutrient levels are related to hydrological connectivity. For instance, river water, being colder and containing higher concentrations of nutrients, affects floodplain wetlands through surface or groundwater inputs. Therefore, the effects of hydrological connectivity on macroinvertebrate abundance may be more substantial, indirectly, than suggested by the statistical analyses conducted in this study, due to the additional correlations with other environmental variables (Zimmer *et al.* 2000). In fact, if more environmental variables potentially inter-correlated with hydrological connectivity were included in our CCA analysis, the importance of the hydrological gradient might be further reduced (Peres-Neto *et al.* 2006). We conclude that aquatic communities and environmental conditions are driven by a common hydrological gradient, which generates complex relationships between them (Legendre and Troussellier 1988).

Besides community composition, macroinvertebrate attributes differed among wetland hydrological types. In our study, invertebrate richness and total abundance increased with hydrological connectivity, whereas invertebrate diversity and evenness showed a rather unimodal pattern. The response of macroinvertebrate diversity is consistent with the Intermediate Disturbance Hypothesis (Connell 1978), since it predicts highest diversification of aquatic communities at intermediate levels of disturbance. This is based in the idea that at low disturbance, competitive exclusion by the dominant species arises; whereas at high disturbance, only species tolerant of the stress can persist. Other authors have reported an increase in invertebrate richness with hydrological connectivity, which peaked at intermediate-flooded sites (e.g., Ward 1998, Tockner *et al.* 1999b, Amoros and Bornette 2002, Ward *et al.* 2002, Whiles and Goldowitz 2005). Most of these studies indicated that hydrological connectivity produces complex effects even within the same water-body, but that hydrological connectivity provides complementary habitats that are required by different life stages of some species (Amoros and Bornette 2002).

Beyond hydrological connectivity, salinization and nutrient status were also found to influence invertebrate patterns across the river floodplain. Certainly, both gradients commonly are known to influence aquatic communities directly or indirectly (e.g., Blumenshine *et al.* 1997, Tockner *et al.* 1999a, Jeppesen *et al.* 2003, Wang *et al.* 2007). The influence of nutrient status on aquatic communities was further reflected in GAM models, which showed significant trends of richness, abundance and diversity along the nitrate gradient. Nitrate is the most common dissolved form of nitrogen in agricultural wastewaters and is often used to assess water quality and ecosystem ecological integrity (Smith *et al.* 2007). The response of macroinvertebrate attributes against nitrate concentration is surprising, since we expected the diversity of aquatic communities to be negatively affected by intensive agriculture land-use, indicated by high nitrate concentration. Thus, contrary to our expectation, macroinvertebrate diversity was found to peak at high nitrate levels, which may be related to the availability of habitats and food resources in nutrient-rich wetlands.

Factors that might account for some of the unexplained variance in our study are morphometry (e.g., Heino 2000, Jeppesen *et al.* 2003), submerged macrophyte cover (e.g., Carpenter and Lodge 1986), riparian vegetation (e.g., Wissmar 1991), substrate structure and composition (e.g., Griffith *et al.* 2001, Murphy and Davy-Bowker 2005), trophic interactions (e.g., Blumenshine *et al.* 1997, Jeppesen *et al.* 2003) and pollution (e.g., Petridis 1993, Woodcock and Huryn 2007). As significant differences between seasons were reported in environmental characteristics and macroinvertebrate assemblages, temporal variation, which has not been included in multivariate analyses in this study, may also account for some of the unexplained variance.

Effects of wetland creation at the scale of the floodplain

Constructed oxbow lakes in the study area consisted of two ponds recently built to mitigate habitat loss. Ground movements after construction, wind-driven bottom re-suspension and shore scouring may have increased recent salt concentrations, but re-suspension is expected to decline once aquatic plant communities colonize and stabilize the lakes. Also, nitrate concentrations were high in the constructed lakes because there was little vegetation, biofilm or organic sediment layers where uptake and de-nitrification could occur (Bachand and Horne 2000, Saunders and Kalff 2001). In fact, the low chlorophyll-a concentration at these sites is probably associated with high turbidity and general wetland instability. The new habitat formed in the constructed oxbow lakes differed from those already present and was rapidly colonized by macroinvertebrates that had not been documented previously (e.g., *Trithemis annulata, Coenagrion scitulum*). Those new taxa dispersed to old, degraded oxbow lakes within the study area, thus enhancing biological diversity over the whole floodplain.

Concluding remarks

The environmental characteristics of the Middle Ebro floodplain are similar to those of other regulated Mediterranean rivers, which are characterized by highly irregular flows that are caused by the high spatial and temporal variability of the Mediterranean climate (Gasith and Resh 1999). The salinization of Mediterranean rivers is caused by river regulation, pollution and changes in land-use, which reduce water flow and enhance river incision, sediment scouring, and water eutrophication (Ward and Stanford 1995, Ward 1998, Gasith and Resh 1999).

Our results suggest that hydrological connectivity strongly influences the composition of macroinvertebrate assemblages in floodplain wetlands. The complexity of the relationship is substantial and has been observed in other lowland rivers (e.g., Tockner *et al.* 1999b, Sheldon *et al.* 2002, Arscott *et al.* 2003, 2005, Whiles and Goldowitz 2005, Reese and Batzer 2007). Water diversion and levee construction threaten the natural variation in hydrology, which reduces the magnitude, frequency and duration of flows, and potentially reduces frequency and duration of connectivity between water bodies (Dynesius and Nilsson 1994, Heiler *et al.* 1995, Poff *et al.* 1997, Tockner *et al.* 1999b, Ward *et al.* 2002). Consequently, the recruitment and dispersal of flora and fauna, coupled with the exchange of water, nutrients and organic matter between floodplain wetlands and the main channel are inhibited, which has negative repercussions for river ecosystem biodiversity and functionality (Amoros and Roux 1988, Heiler *et al.* 1995, Walker *et al.* 1995). Beyond connectivity, other factors that influence the structure of aquatic communities include transport and accumulation of dissolved salts and micronutrients, which enhance ecosystem productivity, but reduce the ecological integrity of strongly eutrophic sites. As a consequence, water quality management in the river catchment is needed to improve wetland ecological integrity.

The present results provide guidance for the management and restoration of riverscapes, which should take into account the principle forces that drive ecosystem integrity, and should also be designed so as to compensate for the lack of conservation in the study area. In the short term, at least, wetland construction had a positive effect on floodplain habitats and invertebrate diversity. Such findings underscore the importance of increasing the diversity of wetlands in degraded floodplains as a means to facilitate the recovery of the riverine biodiversity.

Chapter 2

Modelling the response of floodplain aquatic assemblages across the lateral hydrological connectivity[2]

Abstract

Hydrological connectivity is one of the main controlling factors of habitats and aquatic assemblages in river floodplains. Nevertheless, the lack of unified measures of river-floodplain connectivity (i.e., the Lateral Hydrological Connectivity, LHC) limits understanding floodplain functioning across different systems, and impedes comparing the response of aquatic assemblages to hydrological connectivity. To address these needs, we tested the ability of six different LHC surrogates to model changes in richness, abundance and composition of aquatic assemblages across a Mediterranean floodplain (Ebro River, NE Spain). As shown by Generalized Additive Models, LHC surrogates explained 17% to 72% of the richness and abundance of aquatic assemblages. Macroinvertebrate richness and abundance were most closely related to flood duration, while variance in zooplankton and phytoplankton richness and abundance were best accounted for by water-level variability. Zooplankton, phytoplankton and macroinvertebrates showed overlapping peaks of richness at flood duration rates of 5, 30 and 40 days yr^{-1} respectively. Redundancy Analyses showed that LHC surrogates explained 17% to 37% of aquatic assemblage composition. Distance to the river and flood duration were the most important determinants of macroinvertebrate composition, whereas flood magnitude and water-level variability best accounted for the variance in zooplankton and phytoplankton compositions. Models based on LHC surrogates such as those presented here can help in predicting the consequences of restoration measures and may be useful in setting restoration goals for aquatic assemblages.

Introduction

The habitats and biological assemblages of river-floodplain ecosystems are structured on different spatio-temporal scales, primarily by river discharge pulses and hydrological gradients across the river-floodplain (Junk 1989, Ward *et al.* 1999, Tockner *et al.* 2000). In the last decades, changes in richness, composition and traits of invertebrate assemblages have been widely investigated in an effort to describe hydrological patterns in river-floodplains, as well as to plan and monitor restoration activities (Schiemer 1999, Reckendorfer *et al.* 2006). Among hydrological connectivity types, upstream-downstream connectivity (i.e. longitudinal gradients) have been the most widely

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studied (e.g., Usseglio-Polatera and Beisel 2002, Reese and Batzer 2007). To a lesser extent, the river-floodplain connectivity (i.e. lateral hydrological connectivity) has also been approached since the floodplain habitat is known to play a key role in riverine processes and biodiversity (Tockner *et al.* 1999b, Amoros and Bornette 2002, Wantzen *et al.* 2008).

The lateral hydrological connectivity (LHC) may have direct and indirect effects over the aquatic biota. For instance, nutrient input related to LHC has a direct effect on primary producers (Van den brink and Van der Velde 1994, Hein *et al.* 2003, Wantzen *et al.* 2008) and indirectly impacts on the food quality and quantity for invertebrate species (Woodward and Hilldrew 2002). The pulsing of the river discharge creates a high spatio-temporal heterogeneity (Wantzen *et al.* 2008) and is important for the dispersal of organisms between inter-connected habitats, thus affecting the distribution of species (Baranyi *et al.* 2002, Frisch *et al.* 2005). Moreover, different groups of aquatic organisms may respond to the same hydrological changes in a different way (Gascón *et al.* 2007). However, only a few studies dealing simultaneously on various groups of organisms exist in floodplain habitats (Van den Brink and Van der Velde 1994) with most of the research focused only on one group (Tockner *et al.* 1999b). For instance, while fish (Ward *et al.* 1999, Sheaves *et al.* 2007) and zooplankton (Frisch *et al.* 2005) richness has been reported to be favoured by a high connectivity, macroinvertebrate species attained their maximum richness in intermediately connected sites (Tockner *et al.* 1999b), amphibians and macrophytes were enhanced by stability in disconnected areas (Tockner *et al.* 1999b), and no significant trends were identified for floodplain vegetation (Pautou and Arens 1994). In contrast, other authors have reported an increase in macrophytes in backwaters permanently connected to the river (Bornette *et al.* 1994), while amphibians richness increased in intermediately connected sites (Joly and Morand 1994) and no significant trends were identified for fish (Persat *et al.* 1994). As most of these authors have reported that hydrological connectivity has different and sometimes contradictory effects even within the same wetland (Amoros and Bornette 2002), it is difficult to know which would be the simultaneous response of different groups of organisms against the same LHC gradient and, moreover, how would they respond to future changes in LHC.

Additionally, one of the main problems that exist when studying the LHC gradient concerns the difficult generalization of local indirect LHC surrogates such as those based on water quality or landscape features, and the high costs of obtaining direct on-site measurements of LHC (Galat *et al.* 1998). Although a number of methods have been developed based on categorical or indirect descriptors as geomorphology, water conductivity, vegetation cover or substrate size (e.g., Arscott *et al.* 2005, Paillex *et al.* 2007), only a few studies have employed descriptors directly related to river flow, usually flood duration or water turnover (e.g., Baranyi *et al.* 2002, Foeckler *et al.* 2006, Reckendorfer *et al.* 2006). Thus, the identification of a unified surrogate to reliably estimate the LHC gradient is a long-standing goal needed to compare the response of different floodplains to river pulses and associated hydrological gradients.

Once the key role of hydrological connectivity was proved in general terms for macroinvertebrate assemblages in Chapter 1, information on hydrological variations and other aquatic groups was used to farther assess the effect of hydrological connectivity on the aquatic food web. To address this need, the present study tested the ability of six different LHC surrogates to model patterns in biological features of floodplain aquatic assemblages in a regulated but still dynamic floodplain (the Ebro floodplain, NE Spain). Accurate LHC surrogates were extracted from aerial photographs, a Digital Elevation Model (DEM) and continuous water-level data on a pool of representative wetlands. Floodplain aquatic assemblages studied included macroinvertebrates, zooplankton and phytoplankton. Specifically, our main objectives were (i) to model the biological response (in terms of abundance and richness) of floodplain aquatic assemblages to LHC surrogates and, (ii) to investigate how the aquatic assemblage composition changes across the LHC gradient. In this sense, we expected aquatic richness and abundance to be generally favoured by hydrological connectivity, which increases nutrient inputs and the possibilities of colonization from the main river channel (Lepori and Hjerdt 2006).

Materials and methods

Selected sites for this Chapter included three natural oxbow lakes (NOLs) (Fig. 5), corresponding to the stretch-scale sampling described in General Methodology. Site selection was restricted to these three sites because hydrological and plankton data were only available for these three NOLs and not for COLs or RS.

Triplicate macroinvertebrate, zooplankton and phytoplankton samples were collected with a bimonthly frequency during 2006 in the three NOLs (3 sites x 3 replicates x 6 times= 54 samples). Macroinvertebrate sampling and identification was described in General Methodology. Macroinvertebrate attributes used in this study included richness (MR; number of taxa) and abundance (MN; catches per unit effort, CPUE).

Zooplankton samples were obtained by filtering 5 L of water through a 50-µm net, and the material retained in the net was preserved in the field in 5% formalin. Phytoplankton samples were obtained by directly collecting a 50 µm-filtered sample that was fixed in the field with Lugol’s solution. Both zooplankton and phytoplankton samples were later identified to the genus level using an inverted microscope (NIKON-Eclipse®) with the exception of copepods, ostracods and diatoms which were analyzed to order level. Plankton attributes included richness (ZR and PN; number of taxa) and abundance (ZN and PN; number of individuals L^{-1}).

LHC surrogate descriptors

Fluctuations in river discharge during 2006 allowed us to cover different hydrological situations in the floodplain, ranging from an extraordinary flood (1586 m^3 s^{-1}, 2-yr return period) in February that connected all study sites to the river channel, to the summer minimum (22.4 m^3 s^{-1}) when the disconnection between the river and sites in the floodplain under study was maximal. Six hydrological descriptors were chosen to potentially affect aquatic assemblages (Table 8). They integrated different spatial and temporal scales, were continuous variables directly related to river discharge and so were used as LHC surrogates:

- River distance (RD): minimum distance (m) from each sampling point within a wetland to the closest point at the river channel, calculated from aerial photographs.
- Flood duration (FD): number of days that the river flow rose above the flood-limit (i.e., river flow that connects a particular NOL with the river by surface pathways) in a 365-day period before each sampling date. We calculated flood limits from a detailed digital elevation model (DEM) and contrasted them with field measurements. The three NOLs were flooded sequentially as river flow increased: NOL1 became surface connected at 400 m^3 s^{-1}, NOL2 at 800 m^3 s^{-1}, and NOL3 at 1200 m^3 s^{-1}. The same FD value was assigned to the three replicates collected in each NOL.
- Flood magnitude (FM): increase in water-level relative to the minimum water-level in the study period. FM was calculated as:

  $$FM\ (cm) = (WL - WL_{min})$$

  where WL is the water-level of a particular NOL, and WL_{min} is the minimum water-level in the studied period. Water-level in each NOL was recorded continuously using pressure-based meters with 1-cm resolution (DI-241 Diver, Van Essen Instruments®). One data logger was located at centre of each NOL, thus the same value of FM was assigned to the three replicates collected within each NOL.
- Water-level variability (WLV): coefficient of variation of the water-level over a period of 7 (WLV-7), 180 (WLV-180) or 365 (WLV-365) days before each sampling date. WLV was calculated as:

$$WLVn(\%) = \frac{SDn_{WL} \cdot 100}{\left(\sum_{1}^{n} WL_n \middle/ n\right)}$$

where SDn_{WL} is the standard deviation of water-level (WL) corresponding to a period before the sampling date (n) of 7, 180 or 365-days, respectively. Thus, 3 LHC surrogates were calculated depending on extend of the period used: WLV-7, WLV-180 and WLV-365. Water-level variability was considered the same for the three replicates collected in each NOL.

Table 8 General features and mean (SD) values of LHC surrogates and biological attributes in each studied site.

	Variable, Abbreviation (units)	**NOL1 (n=18)**	**NOL2 (n=18)**	**NOL3 (n=18)**
General	Age (yrs)[A]	42	61	61
	Area (ha)[B]	70.31	35.45	10.33
	Minimum depth (cm)[C]	38.29	50.91	86.92
	Maximum depth (cm)[C]	650.05	351.20	439.65
	Flood limit ($m^3 s^{-1}$)	400	800	1200
Biological attributes	Macroinvertebrate	11.33	8.1	6.34
	Richness, MR (nº taxa)	(4.45)	(2.60)	(2.53)
	Macroinvertebrate	642.64	122.80	282.01
	Abundance, MN (CPUE)	(476.75)	(163.12)	(316.60)
	Zooplankton	4.21	2.57	4.36
	Richness, ZR (nº taxa)	(1.53)	(1.15)	(2.20)
	Zooplankton	965.76	307.23	2832.58
	Abundance, ZN (nº L^{-1})	(1619.89)	(605.60)	(2764.62)
	Phytoplankton	7,00	6,10	7,47
	Richness, PR (nº taxa)	(2,78)	(1,54)	(2,00)
	Phytoplankton	12025	81303	57527
	Abundance, PN (nº mL^{-1})	(11187)	(13737)	(39217)
LHC surrogates	Flood Duration, FD	40.71	6.00	2.50
	(days yr^{-1})	(5.89)	(2.07)	(1.15)
	Flood Magnitude, FM	57,78	16,83	47,28
	(cm)	(44,58)	(10,87)	(18,06)
	Water-level Variability	16,76	13,25	5,41
	in 7 days, WLV-7 (%)	(11,93)	(21,44)	(8,70)
	Water-level Variability	44.88	26.65	10.68
	in 180 days, WLV-180 (%)	(18.04)	(15.51)	(5.53)
	Water-level Variability	58,98	32,12	11,00
	in 365 days, WLV-365 (%)	(4,67)	(4,77)	(2,14)
	River Distance, RD	1053,00	993.33	791.33
	(m)	(86,12)	(391.41)	(256.74)

[A] Age is measured since the creation of oxbow lakes

[B] Area was measured with aerial photographs in summer.

[C] Minimum and maximum depths from January 2005 to December 2006.

Statistical analyses

The response of biological attributes to the LHC was assessed using regression models. The Generalized Additive Model (GAM, Wood 2006) was chosen because it has been successfully used for species modelling in relation to environmental variables (Castella *et al.* 2001) and is a flexible tool that allows both linear and smoothing methods to be applied. Response variables in the models included biological attributes of macroinvertebrates, zooplankton and phytoplankton while explanatory descriptors included LHC surrogates. However, given that river distance, flood duration, flood magnitude and water-level variability are closely related, a high level of collinearity was expected among them. Therefore, to avoid this problem, a pre-selection of LHC surrogates was made by means of Spearman correlation analyses (Castella *et al.* 2001) (Table 9). We thus selected RD, FD, FM and WLV-7 that had $r_S < 0.5$ as a set of less correlated surrogates that will be used as explanatory variables in GAM models. Other arguments used in GAM models included: (1) a quassipoisson family distribution for the response variables (biological attributes), (2) three maximum degrees of freedom to reduce the complexity of the models (Castella *et al.* 2001), (3) stepwise backward selection of explanatory variables to retain only the most significant explaining the response of the biological attribute, and (4) a likelihood ratio-test to check the significance of each step of the backward selection (Crawley 2002). The percentage of deviance explained was used to assess the goodness-of-fit of the final model. The contribution of each explanatory variable to the final model was tested by evaluating the drop in deviance explained (drop-contribution) by the model when the explanatory variable was removed, and so a high drop-contribution implies a high contribution of the variable, since its deletion cause a high reduction of the deviance explained by the model (Castella *et al.* 2001). GAM analyses were performed with package "mgcv" (Wood 2006) in R 2.5.1 software (R Development Core Team 2007). Functions used to that end can be consulted in Appendix 4.

Table 9 Spearman correlations (r_S) among LHC surrogates used in this study. LHC surrogates with $r_S < 0.5$ were selected for regression models.

	Area	**RD**	**FD**	**FM**	**WLV-7**	**WLV-30**	**WLV-180**	**WLV-365**
Area	1							
RD	0.48*	1						
FD	0.88*	0.44*	1					
FM	0.03	0.07	0.26	1				
WLV-7	0.46*	0.23	0.66*	0.43*	1			
WLV-30	0.46*	0.21	0.60*	0.32*	0.89*	1		
WLV-180	0.75*	0.36*	0.71*	0.13	0.57*	0.60*	1	
WLV-365	0.94*	0.46*	0.90*	-0.02	0.55*	0.56*	0.78*	1

* Significant correlation at $P < 0.05$

In addition, a multivariate approach was used to assess the response of the aquatic assemblage composition to the LHC gradient. To that end, we performed Redundancy Analyses (RDA) between the taxa abundance of each set of species (macroinvertebrates, zooplankton and phytoplankton) and the complete set of LHC surrogates. The three species sets were centred and standardized by species as recommended by ter Braak and Šmilauer (2002). To avoid collinearity problems, a forward-selection procedure available in CANOCO 4.5 was used to select non-redundant variables (using a cut-off point of 0.1; Magnan 1994). Additionally, the inflation factor of selected variables was checked to be less than 10, ensuring a minimum redundancy among the retained variables (ter Braak and Šmilauer 2002). A Monte Carlo Permutation Test was used to assess the significance of the first canonical axis as well as the full model (Hope 1968). By means of these RDA analyses, we were able to identify the most significant LHC surrogates structuring each aquatic assemblage and to achieve the ordination of species in the factorial space defined by the selected LHC surrogates. In addition, RDA analyses allowed us to compare the selected LHC surrogates among the different aquatic assemblages tested. RDA analyses were performed with CANOCO 4.5 (Microcomputer Power, Ithaca, New York).

Results

Response of aquatic assemblage richness and abundance to LHC surrogates

The Generalized Additive Models (GAM) relating biological attributes to LHC surrogates were significant and showed the complex responses of aquatic assemblages to hydrological patterns (Figs. 14, 15 and 16). Goodness-of-fit of the models ranged from 17% to 72%, with macroinvertebrate attributes being better accounted for by LHC surrogates (deviance explained > 60%) than zooplankton and phytoplankton attributes (deviance explained < 60%) (Table 10).

Table 10 Results of regression models (Generalized Additive Models) performed between biological attributes (response variables) and LHC surrogates (explanatory variables). The % of deviance explained by each model is shown (Total expl.). The contribution of each descriptor to the model is measured by the drop in the deviance explained by the model when the descriptor is removed (Drop-contribution). Variables abbreviations are explained in the text and Table 8.

	Drop-contribution (%)				**Total expl. (%)**
	RD	**FD**	**FM**	**WLV-7**	
MR	22.4	39.7	13.6	6	72.2
MN	7.4	32.9	-	5.2	64.7
ZR	-	16.6	7.5	17.9	58.8
ZN	12.1	20.1	-	14	51.1
PR	-	17.4	-	-	17.4
PN	-	-	15.9	41.87	43.9

-: non-significant LHC surrogate at $\alpha = 0.05$

Macroinvertebrates— Macroinvertebrate Richness (MR) was significantly related to river distance (RD), flood duration (FD) and flood magnitude (FM), and increased linearly with seven-day water-level variability (WLV-7). As shown in Table 10, FD accounted for the highest drop-contribution among LHC surrogates. The model showed that both MR and MN increased under conditions where FD was 10–40 days yr^{-1} (Fig. 14). Macroinvertebrate abundance (MN) exhibited a complex cubic response to RD and FD, and increased linearly with WLV-7. Again, FD showed the highest drop-contribution to the model.

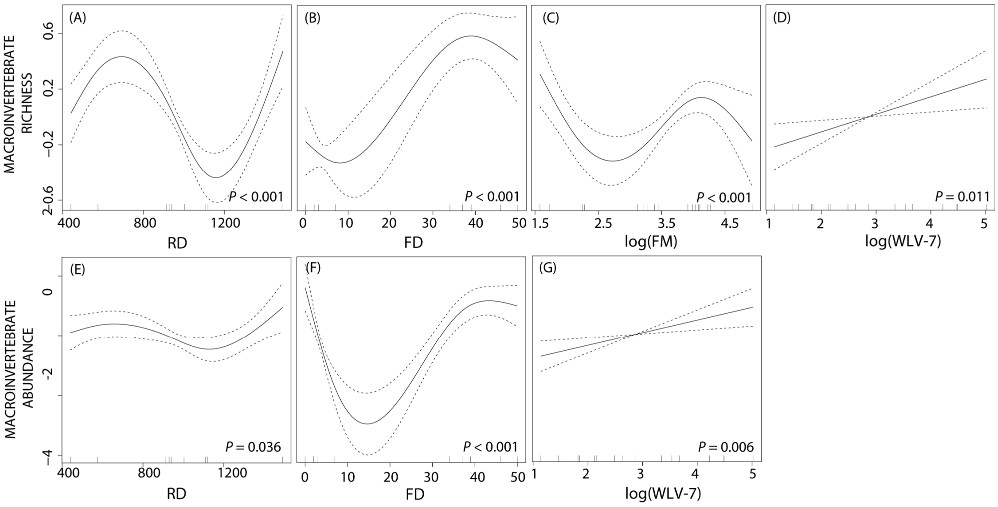

Figure 14 Response of macroinvertebrate richness (A-D) and abundance (E-G) to LHC surrogates. Modelling is based on Generalized Additive Models (GAM) with stepwise selection of surrogates. Response variables are represented on the y-axis as a smoothing function, and thus are dimensionless. Samples location is displayed in the x-axis (upper ticks). The dashed lines represent 95% confidence intervals around the smoothed function. For a further description of variables and GAM modelling, see text.

Zooplankton— Zooplankton richness (ZR) was lowest at intermediate flood duration (10-20 days yr^{-1}) and reached highest values under short duration of floods, between 0-10 days yr^{-1}. In contrast, ZN varied inversely with FD, with high ZN associated with low FD and lower ZN associated with high values of FD (Fig. 15).

Phytoplankton— Phytoplankton richness (PR) was significantly related only to FD (Table 10), exhibiting an increase at 20-30 days yr^{-1} of FD, thus conversely to patterns found for ZR (Fig. 15). Phytoplankton abundance (PN) generally increased with increasing values of FM but was negatively related to WLV-7 (Fig. 16). Removing WLV-7 from the model caused the highest drop in deviance explained (42%) by any model (Table 10). The response of PN to both FM and WLV-7 was similar to the response of ZN to the same hydrological gradients (Figs. 15 and 16).

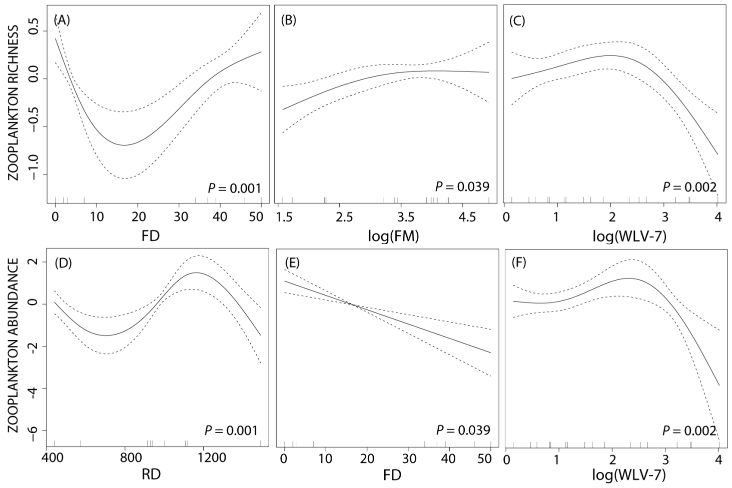

Figure 15 Response of zooplankton richness (A-C) and abundance (D-F) to LHC surrogates. Modelling is based on Generalized Additive Models (GAM) with stepwise selection of surrogates. Response variables are represented on the y-axis as a smoothing function and thus are dimensionless. Samples location is displayed in the x-axis (upper ticks). The dashed lines represent 95% confidence intervals around the smoothed function. For a further description of variables and GAM modelling, see text.

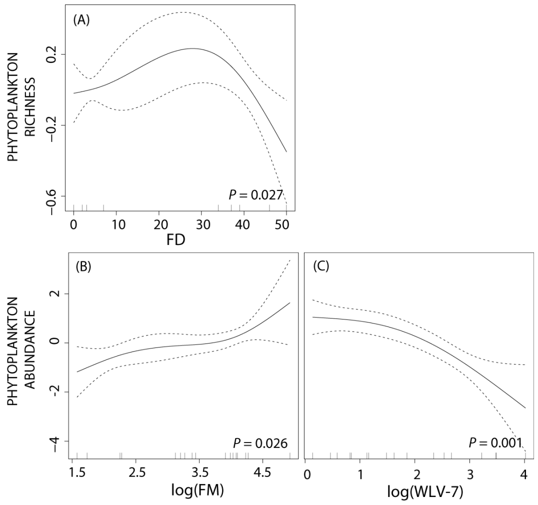

Figure 16 Response of phytoplankton richness (A) and abundance (B-C) to LHC surrogates. Modelling is based on Generalized Additive Models (GAM) with stepwise selection of surrogates. Response variables are represented on the y-axis as a smoothing function and thus are dimensionless. Samples location is displayed in the x-axis (upper ticks). The dashed lines represent 95% confidence intervals around the smoothed function. For a further description of variables and GAM modelling, see text.

To summarize, FD was included in five out of six models, and accounted for the highest drop-contribution in four (MR, MN, ZN and PR, Table 10). However, the wide confidence intervals in FD plots evidenced a lack of accuracy in predictions made in the range of 10–40 days yr^{-1}. The lack of representative wetlands for this FD range is likely responsible, at least in part, for this lack of accuracy. Another LHC surrogate, WLV-7, was included in five models, but represented the highest drop-contribution in only two cases (ZR and PN).

Finally, the opposite patterns of macroinvertebrate and plankton attributes with respect to LHC surrogates should be highlighted. While this opposing relationship is especially clear in the case of

MR and PR against FD, and MN and PN against WLV-7, most macroinvertebrate and zooplankton attributes showed contrasting trends against all LHC surrogates (Figs. 14, 15 and 16).

Response of aquatic assemblage composition to LHC surrogates

Redundancy Analysis (RDA) relating the composition of macroinvertebrate, zooplankton and phytoplankton assemblages to the set of LHC surrogates were highly significant and explained 17% to 37% of aquatic assemblage composition.

Macroinvertebrates—RDA1 (Monte Carlo Permutation Test of first axis, F-ratio = 7.44, $p < 0.001$; of all canonical axes, F-ratio = 4.95, $p < 0.001$) linked macroinvertebrate assemblage composition to the LHC surrogates, selecting FD and RD among the six surrogates. FD correlated with the first axis, whereas RD was related to the second axis. Together, FD and RD explained 17% of macroinvertebrate variability. However, only the first axis corresponding to flood duration, which explained 14% of macroinvertebrate variability, was considered for interpretative purposes. Different macroinvertebrate taxa were favoured by the different connectivity situations. For instance, aquatic insects (e.g., *Caenis* sp., *Ecnomus* sp., Chironomidae) and aquatic worms (e.g., *Nais* sp.) were more abundant under long-duration flood conditions (high FD values), whereas crustaceans (*Atyaephira* sp. and *Echinogammrus* sp.) increased under conditions where flood events were shorter (low FD values) (Fig. 17A).

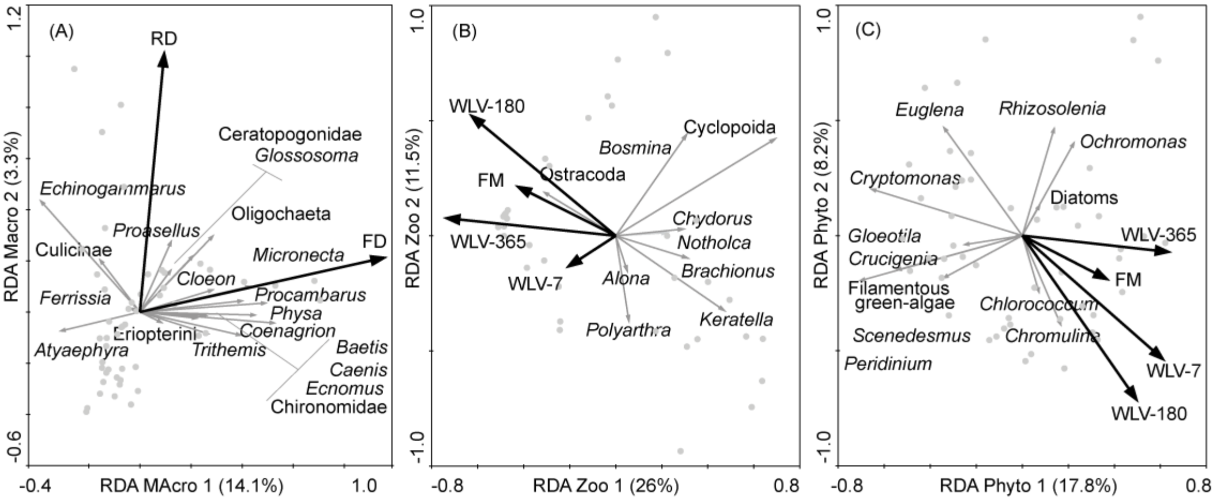

Figure 17 Results of Redundancy Analysis (RDA) performed between the macroinvertebrate (A), zooplankton (B) and phytoplankton (C) assemblages composition and LHC surrogates (stepwise selection of LHC surrogates with $p < 0.1$ and inflation factor < 10). Black arrows indicate the direction of the steepest increase in the LHC surrogate. Gray arrows indicate the direction of the steepest increase in the values of the corresponding species abundance.

Zooplankton—RDA2 (Monte Carlo Permutation Test of first axis, F-ratio = 15.82, $P < 0.001$; of all canonical axes, F-ratio = 7.06, $P < 0.001$) related zooplankton assemblage composition to four of the six potential LHC-surrogates. These variables (FM, WLV-7, WLV-180 and WLV-365) were grouped at the left side of the plot and collectively explained 37% of zooplankton variability. Zooplankton taxa were grouped towards the right side of the plot, thus in the opposite direction of the LHC surrogates (Fig. 17B). Therefore, in general, an increase in all LHC surrogates implies a decrease in zooplankton taxa abundance. The only exception to this pattern was the ostracods, which increased with higher values of water-level variability and flood magnitude (Fig. 17B).

Phytoplankton— Similar to the RDA of zooplankton taxa, RDA3 (Monte Carlo Permutation Test of first axis, F-ratio = 9.75, $P < 0.001$; of all canonical axes, F-ratio = 5.24, $P < 0.001$) related the phytoplankton assemblage composition with the LHC surrogates FM, WLV-7, WLV-180 and WLV-365, which collectively explained 32% of phytoplankton variability. These surrogates were grouped towards the right side of the plot while most of phytoplankton taxa were grouped on the opposite side (Fig. 17C). Conditions of high water-level variability and high flood magnitude (high values of WLV and FM) were related to increases in Chrysophyceans (e.g., *Ochromonas* sp., *Chromulina* sp.) and diatoms (e.g., *Gyrosigma* sp., *Nizschia* sp.), whereas low water-level variability and low flood magnitude (low values of WLV and FM) were associated with greater abundance of Chlorophyceans (e.g., *Scenedesmus* sp., *Crucienia* sp.), Dinophyceans (e.g., *Peridinium* sp.), Euglenoids (e.g., *Euglena* sp.) and Cryptophyceans (e.g., *Cryptomonas* sp.).

In summary, RDA analyses demonstrate a contrasting response to LHC of macroinvertebrates on the one hand, and zooplankton and phytoplankton on the other. LHC surrogates selected in RDA1 (RD and FD) were different from those selected in RDA2 and RDA3 (FM, WLV-7, WLV-180 and WLV-365). Notably, although WLV-7, WLV-180 and WLV-365 were highly correlated (Table 9), they were retained in RDA2 and RDA3 analyses because they had inflation factors < 10. In addition, while LHC surrogates selected by each RDA were generally grouped in the first axis, macroinvertebrate taxa were grouped in the same direction as LHC surrogates, whereas zooplankton and phytoplankton taxa were grouped in the opposite direction (Fig. 17). These results indicate that an increase in LHC-surrogate values is associated with a general increase in macroinvertebrate taxa abundance but a decrease in zooplankton and phytoplankton taxa abundance.

Discussion

Response of aquatic assemblage richness and abundance to LHC

As expected, the attributes of the aquatic assemblages were significantly related to the LHC surrogates tested, as shown by GAM models. These models revealed a complex response of macroinvertebrate assemblage attributes, which were generally favoured by LHC, and zooplankton and phytoplankton attributes, which were generally favoured by stability and lack of LHC. Zooplankton, phytoplankton and macroinvertebrate attributes showed consecutive peaks of richness at flood duration rates of 5, 30 and 40 days yr^{-1} respectively. Moreover, the goodness-of-fit of the models showed that macroinvertebrate attributes were more closely related to LHC surrogates than were zooplankton and phytoplankton attributes.

Macroinvertebrates—The effect of disturbance on aquatic invertebrate attributes depends on the balance between removal of organisms during floods and subsequent re-colonization (Lepori and Hjerdt 2006). However, the frequency of high-magnitude events in the Ebro floodplain is limited by river regulation and embankment. For that reason, we expected macroinvertebrate attributes to be positively related to an increase in LHC, which allows opportunistic species to colonize wetlands through passive or active aquatic dispersal (Jenkins and Boulton 2003). Certainly, in agreement with other studies (e.g., Tockner *et al.* 1998, Whiles and Goldowitz 2005, Reckendorfer *et al.* 2006, Paillex *et al.* 2007), we found that macroinvertebrate richness and abundance was highest under conditions of intermediate to high flood-duration rates (30-40 days yr^{-1}). Previous studies in the Ebro floodplain related this fact to nutrient enrichment caused by the input of river water during floods, which may enhance the growth of opportunistic species (Gallardo *et al.* 2008).

Zooplankton— Hydrological connectivity is usually known to enhance primary productivity and plankton diversification through nutrient inputs and enhanced passive dispersal. This is especially true in temporal habitats where floods connect dried-out habitats thereby triggering the aquatic productivity (Jenkins and Boulton 2003, Frisch *et al.* 2005). In contrast, we found that among permanent floodplain wetlands exhibiting different degrees of hydrological connectivity with the Ebro River, zooplankton richness and abundance peaked in the most hydrologically stable and isolated sites (flood duration of 0-10 days yr^{-1}). Thus, our results are generally in accord with those of Baranyi *et al.* (2002), who suggested that frequent and intensive floods may reduce the zooplankton assemblage — for example, through drift, wash-out or lack of refuge — and diminish the capacity of the zooplankton assemblage to resist further disturbances. The observed decrease in zooplankton taxa abundance under conditions of high frequency and magnitude of floods revealed by the RDA analysis lends support to this idea. In addition, biotic interaction through predation and competition, which have been shown to increase in disconnected habitats (Wellborn *et al*. 1996), could also explain the observed response of zooplankton, opposite to that of macroinvertebrates.

Phytoplankton— The effect of LHC on phytoplankton richness and abundance depends on the balance between wash-out of organisms, increased nutrient concentration and turbidity (Heiler *et al.* 1995). In the Ebro floodplain, phytoplankton richness was highest under intermediate-LHC conditions (20-30 days yr^{-1}) and decreased when flood duration exceeded 30 days yr^{-1}. In this context, Van der Brink and Van der Velde (1994) suggested that high connectivity often results in hypereutrophic conditions, and thereby reduces phytoplankton richness. Likewise, nutrient accumulation due to confinement under low-LHC conditions in the Ebro floodplain (Gallardo *et al.* 2008) lead to a hypereutrophic situation that may result in a decrease in phytoplankton richness as well.

Response of aquatic assemblage composition to LHC surrogates

Results from multivariate (RDA) analyses further demonstrated the contrasting effects of hydrological connectivity on the composition of macroinvertebrate, zooplankton and phytoplankton assemblages. While macroinvertebrate composition appeared to be positively influenced by closeness to the river and the duration of floods, the plankton assemblages seemed to be negatively related to flood magnitude and fluctuations in the water-level. RDA analyses revealed that the following changes in species composition significantly depended on LHC conditions:

Macroinvertebrates— The composition of invertebrate assemblages was mainly structured by the duration of floods, which is known to be related to the re-colonization of disturbed habitats (Lepori and Hjerdt 2006). In this sense, an increase in aquatic insects and aquatic worms with increasing LHC has been observed, consistent with previous studies performed in the Ebro floodplain (Gallardo *et al.* 2008) as well as other floodplains (e.g., Juget and Lafont 1994). In contrast, low-LHC conditions associated with short-duration flood events favoured aquatic organisms such as crustaceans and gastropods, which, because of their long life-cycles and low effective colonization strategies, are widely known to inhabit stable habitats (Gasith and Resh 1999, Reckendorfer *et al.* 2006). An exception is the crustacean *Procambarus clarkii,* which appeared in frequently connected sites that provide the high-productivity system required this specie (Angeler 2001).

Zooplankton— Ostracods dominated under high-LHC conditions, while copepods, cladocerans and rotifers were more likely to occur under low-LHC conditions. In this sense, Davidson *et al*. (2000) suggested that high phytoplankton densities under low-LHC conditions combined with low current velocities may sustain a high abundance of herbivorous zooplankton and ultimately of carnivorous cyclopoids. Different patterns in the abundance of the broad taxonomic groups may be attributable to habitat characteristics indirectly related to LHC, such as water quality and biological interactions with macroinvertebrates and fish (James *et al.* 2008). Indeed, highest phytoplankton productivity and lowest macroinvertebrate abundance found under low-LHC conditions may have foster the zooplankton community.

Phytoplankton— Diatoms and Chrysophyceans appeared under high-LHC conditions, while Dinophyceans, Chlorophyceans and Euglenoids seemed to be positively related to low-LHC conditions. This distribution of phytoplankton taxa contrasted with previous studies relating the abundance of Chlorophyceans and Euglenoids to nutrient inputs after floods, and linking the abundance of diatoms to higher silicate concentrations in disconnected floodplain lakes (Van den Brink *et al.* 1994). One explanation for these discrepancies is an increase in turbidity after flood events that may limit Chlorophyceans development under high-LHC conditions. Additionally, abundant organic nutrients, which are more likely to accumulate in the confined wetlands of the Ebro floodplain (Gallardo *et al.* 2008), may enhance Dinophyceans and Euglenoids, which are mixotrophic species that are known to exploit high organic concentrations (Jones 2000). Lastly, higher turbulence at high-LHC sites may enhance diatoms by preventing them from sinking (Ferris and Lehman 2007).

Finally, we stress the fact that LHC surrogates explained almost twice as much plankton-composition variance as macroinvertebrate-composition variance in RDA analyses. This is in contrast to GAM models, which showed that LHC surrogates better accounted for macroinvertebrate attributes than plankton attributes. It thus follows that LHC has a stronger effect on macroinvertebrate abundance and richness, as well as zooplankton and phytoplankton taxa composition. Previous studies that simultaneously addressed the responses of plankton and macroinvertebrates to hydrological changes have also found that the two assemblages responded differently to the same disturbance (Gascón *et al.* 2007). Such differences in the responses of macroinvertebrate and plankton assemblages can be related to differences in body size (Dickie *et al.* 1987), habitat use or life-history strategies (e.g., current preference, resistance form, dispersal). Further research, however, is needed to better understand the key environmental factors that affect plankton structure in such a complex system.

Hydrological descriptors most representative of the LHC gradient

Taking the results of GAM and RDA analysis into account, we conclude that there is not a unique LHC surrogate able to reflect the response of every group of aquatic organisms at the same time. Flood duration was the best explaining most of the biological features analysed, although variations in the water-level played an important role, especially for the plankton assemblage composition. Therefore, the election of a LHC surrogate will depend on the group of organism analysed and the study objectives.

Flood duration can be measured with great accuracy by expensive methods, for example with data loggers. But flood duration can also be measured using simpler and cheaper methods, such as Digital Elevation Models (DEM) coupled with flow models or direct measurements in the field, in cases where less accurate estimates are adequate. Flood duration is also a reliable measure that has previously been used successfully, for example in the Danube (Reckendorfer *et al.* 2006), the Upper Rhine (Van den Brink and Van der Velde 1994) and the Elbe (Foeckler *et al.* 2006) floodplains, where the hydrological gradients were greater than those of the Ebro. In contrast, water-level variability can only be measured with data loggers, which are expensive and difficult to install, thus limiting its practical application. Moreover, as long as we know, water-level variability has not been used before to assess patterns in floodplain aquatic assemblages.

Given the high costs of obtaining direct on-site measurements of hydrological connectivity (Galat *et al.* 1998), the identification of key surrogate descriptors capable of reliably estimating the response of aquatic assemblages in the Ebro floodplain is one of the most important results of this study. Furthermore, the comparison between these and other floodplains by means of a unified LHC measure, such as flood duration, could offer an opportunity to better understand the role of hydrological connectivity in important physic, chemical and biological processes.

Implications for river-floodplain management

Over the last century, the natural flow regime in the Ebro River has been modified by progressive regulation of river flow, channel embankments and changes in land-use (Pinilla 2006). Actually, the Ebro River partially maintains its dynamism in its middle stretch, but trends in floodplain degradation suggest that without restoration and recovery efforts, ecosystem function and biodiversity could be lost (Gallardo *et al.* 2008).

Empirical models based on LHC surrogates, such as those presented here, can be helpful in predicting the consequences of restoration measures for aquatic assemblages and thus can be used in setting restoration goals (Reckendorfer *et al.* 2006). For instance, based on the models presented here, a flood connection of 0-10 days yr^{-1} would favour zooplankton richness, while 20-40 days yr^{-1} would enhance macroinvertebrate and phytoplankton richness. Collectively, the diversification of the hydrological conditions in the Ebro floodplain, especially in the range between 10 and 40 days yr^{-1}, would be useful to diversify the habitats and aquatic assemblages of the floodplain. A diverse range of hydrological connectivity can be restored at a local scale by combining different restoration activities as managing the river discharge, reconnecting disconnected waterbodies, and lowering the floodplain embankments (Buijse *et al.* 2002).

By showing a clear relationship between the duration of floods and the variation of the water-level on the one hand and the richness, abundance and composition of aquatic assemblages on the other, this study can facilitate floodplain conservation and restoration in the Ebro River in two important ways. First, it offers a means to reduce the costs, in terms of both time and money, required for floodplain LHC monitoring. Second, it provides a useful tool for predicting the impact on aquatic assemblages of restoration activities directed to change the LHC gradient on a local scale. The efficiency of such techniques to recover a particular group of organisms will depend on a comprehensive knowledge of LHC effects such as the presented here.

Chapter 3

Main environmental factors determining the functional composition and diversity of aquatic communities across the lateral hydrological connectivity[3]

Abstract

The aim of the present study was to describe the relationships between macroinvertebrate traits and lateral hydrological and environmental gradients in a Mediterranean river-floodplain, from the main river channel to three disconnected floodplain wetlands. Bimonthly water and macroinvertebrate samples were collected from an array of riverine wetlands in the Ebro River in northeast Spain. Our analysis of trait structures in wetlands aligned along the lateral hydrological connectivity showed that community composition changed from a rich and abundant invertebrate community dominated by generalist species adapted to disturbance at the river site to a more trait-diversified community of specialist species adapted to stability and biotic interaction in hydrologically disconnected floodplain sites. The diversity of functional groups peaked at intermediate-connected sites, where both generalist and specialist species coexist. The highest richness of functional groups was found in the river site, reflecting its highest habitat heterogeneity. The main environmental variables shaping the structure of invertebrate traits extracted after Redundancy Analysis were flood duration and frequency (surrogates of hydrological disturbance), dissolved solids (surrogate of confinement) and nitrate concentration (surrogate of agricultural pressure). These environmental variables explained 43% of the existing variability in invertebrate traits. Because groups with similar traits responded in different ways to the main gradients, we conclude that the functional grouping of invertebrates provides an adequate and simple tool to assess changes in functionality and the effect of lateral gradients across a floodplain. Our results highlight the need to integrate the study of rivers and their floodplains to better account for their close interaction.

Introduction

Aquatic community structure is determined by both abiotic factors, such as hydrological disturbance and water chemistry, and biotic effects resulting from ecological interaction, principally competition and predation (Batzer and Wissinger 1996, Wellborn *et al.* 1996). Abiotic factors determine the potential habitat available for a particular species while biotic interactions determine a species' actual distribution (Wellborn *et al.* 1996). Abiotic factors, in turn, may reflect natural gradients, such as climate, geology or hydrology, or may be the result of human alterations, such as river

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embankments, and agricultural, urban or industrial sewage. Life-history strategies and habitat use of organisms inhabiting freshwater systems integrate actual and historical conditions, and are comparable across different taxonomic groups. Thus, these factors provide a unified measure of ecosystem constraints and interaction across communities with different taxonomic composition (Statzner 2001, Gayraud *et al.* 2003). Moreover, biological traits are especially telling with respect to ecosystem functionality, biodiversity and environmental alteration. For these reasons, bio-monitoring tools for assessing river alteration in large European rivers have recently focused on biological traits (e.g., Dolédec *et al.* 1999, Gayraud *et al.* 2003, Bady *et al.* 2005, Statzner *et al.* 2005, Dolédec and Statzner 2007). However, certain problems arise when studying the relationship between functional traits and environmental factors because the number of biological and ecological traits that can be used is high (there are more than 100 trait categories in the bibliography). The classification of species into homogeneous groups showing similar life-histories and behavior is one way to solve this problem, as we would expect them to show the same response to key environmental factors (Statzner 2001).

A number of environmental gradients reflecting hydrological patterns, habitat characteristics and water chemistry have been identified as important for aquatic invertebrates in river-floodplain ecosystems (Junk 1989, Tockner *et al.* 1999b). Floodplain wetlands outside the river habitat (e.g., backwaters, oxbow lakes, temporary ponds) are lentic habitats arranged in a lateral hydrological connectivity that become temporarily lotic during river pulses (Amoros and Roux 1988, Ward and Stanford 1995). The position of wetlands on that hydrological gradient is determined by the distance to the river, groundwater seepage and natural or artificial barriers to river flow (Amoros and Bornette 2002). Dissolved salts and organic nutrients introduced by groundwater seepage, runoff and autogenic processes, accumulate in disconnected floodplain wetlands (i.e., those with low connectivity) (Tockner *et al.* 1999a), leading to salinization and eutrophication. In addition, as floodplain areas have become extensively occupied, wetlands with low connectivity and high confinement are increasingly vulnerable to the accumulation of pollutants and nutrients generated by human activities. Among these nutrients, nitrate is the most common form in agriculture watersheds and is often used to assess water quality and ecological integrity of wetland ecosystems (Smith *et al.* 2007).

The dynamics governing the interplay of hydrological connectivity with environmental gradients and human pressure on rivers and their floodplains are difficult to measure directly (Galat *et al.* 1998, Tockner *et al.* 2000) and are sometimes seemingly complex (Amoros and Bornette 2002). However it is well known that extant aquatic communities, which exist by virtue of organisms' adaptation to spatial and temporal variability, reflect the historical pattern of disturbance of a given site (Batzer and Wissinger 1996). Accordingly, macroinvertebrate traits are most often used to explore this historical interplay although examples of studies focusing on aquatic vegetation traits (e.g., Bornette *et al.* 1994, Pautou and Arens 1994, Henry *et al.* 1996) and fish traits (e.g., Persat *et al.* 1994, Poff and Allan 1995) can be found. Examples of representative traits of macroinvertebrates that provide valuable information about how a community adapts to disturbances, either natural (e.g., river pulses) or anthropogenic (e.g., pollution), include potential size, life-span, number of reproductive cycles per year, resistance body-form and attachment to substrate, all of which potentially allow organisms to better resist disturbances and recuperate after a disturbance (Townsend and Hilldrew 1994, Usseglio-Polatera *et al.* 2000a). In contrast, in stable areas less affected by such disturbances, biotic interactions become increasingly important (Wellborn *et al.* 1996). Consequently, functional traits proposed to change according to wetland characteristics include the proportion of organisms belonging to particular functional feeding groups, modes of reproduction, respiration and locomotion, life-history patterns and drift behavior (Usseglio-Polatera *et al.* 2000a).

Many studies have focused on functional-trait responses to environmental gradients, most of which have studied longitudinal gradients (i.e., differences among sites situated along the river). However, relatively less information on the behaviour of such trait responses as a function of lateral gradients in disconnected floodplain wetlands is available, and most of the existing information on European floodplains is from the Rhône River, in France (e.g., Juget and Lafont 1994, Richoux 1994, Tachet *et al.* 1994, Usseglio-Polatera 1994, Usseglio-Polatera and Tachet 1994, Paillex *et al.* 2007). Consequently, there is an unmet need to integrate the study of river and floodplain habitats to attain a complete understanding of trait patterns and processes along lateral gradients in other floodplains. Such understanding will further help to unify ecological assessment protocols and provide guidelines for managing and restoring river-floodplain ecosystems.

The use of a common classification into functional groups (as that proposed by Usseglio-Polatera et al. 2000a) could be a useful tool to both reduce methodological problems related to the number of traits and facilitate comparison between ecosystems. To date however, the use of such functional classification has not been tested in river-floodplain habitats. To this end, the present study focused on the relationships among environmental variables and functional groups of species in a lateral hydrological connectivity (i.e., from the river channel to isolated floodplain wetlands located further into the floodplain) across a Mediterranean river-floodplain (Ebro River, NE Spain). Our first objective was to identify significant differences in trait composition (i.e., abundance of functional groups), functional richness and functional diversity between wetlands aligned along a connectivity gradient. Based on the Intermediate Disturbance Hypothesis (Connell 1978), we would expect that wetlands located in an intermediate position in the hydrological connectivity gradient would show the highest functional diversity because both generalist and specialist organisms coexist there. On the other hand, a previous study developed in the Ebro basin has highlighted three main environmental gradients as important for aquatic invertebrates: hydrological connectivity, salinization and nutrient status (Gallardo *et al.* 2008). Our second objective was thus to analyze the influence of these environmental gradients on the functional-trait structure of macroinvertebrates inhabiting the Ebro floodplain. Additionally, we tested the suitability of using the functional grouping of invertebrates as bio-indicators of those gradients.

Materials and Methods

Selected sites for this study included three natural oxbow lakes (NOL) and the main river channel (RS) corresponding to the stretch-scale sampling (Fig. 5). Sites were selected to represent the range of hydrological conditions currently affecting this segment of the river-floodplain. Besides, selected NOLs showed similar habitat characteristics in terms of morphology, vegetation and substrate texture. Water and macroinvertebrate samples were taken every two months in 2006 from various locations at the four study sites according to the methods described in General Methodology (stretch-scale sampling).

Functional Grouping of taxa

We used the reference classification of invertebrates inhabiting European rivers developed by Usseglio-Polatera *et al.* (2000a) based on data from 472 genera. This classification includes eight uniform biological groups (from “a” to “h”) based on 11 biological traits, extracted and modified from Tachet *et al.* (2000). Among these traits, the authors highlighted the maximum potential size, reproduction behavior (number of cycles per year, life-span and technique), respiration, locomotion, food source and feeding behavior as indicators of ecosystem stability, food web structure and biological interaction.

No representative species of group “a”, composed of Porifera and Bryozoa, were found; thus the taxa appearing in the Ebro floodplains were classified into seven of these eight biological groups (“b” to “h”), whose composition and representative traits are summarized in Table 11. Only one species, *Trithemis annulata,* was not included in the reference classification of Usseglio-Polatera *et al.* (2000a), it was included with the rest of the dragonflies in group “d”.

Table 11 Functional classification of invertebrates appearing in the Ebro floodplain.

Functional group	Taxa included		Biological traits
Group "b"	*Atyaephira* sp.(CRU) *Procambarus* sp. (CRU) *Echinogammarus* sp. (CRU)	*Corbicula* sp. (BIV) *Unio* sp. (BIV) *Ephoron* sp. (EPH) *Helobdella* sp. (HIR)	Medium to large crawlers or burrowers using various food sources and feeding habits. Ovoviviparity reproduction, aquatic respiration and dispersal.
Group "c"	Tipuloidea (DIP) *Hexatomini* (DIP) *Limoniini* (DIP) *Ceratopogonidae* (DIP)	*Ephemera* sp. (EPH) *Erpobdella* sp. (HIR) *Rhyacopila* sp. (TRI)	Medium size, monovoltine crawlers. Cemented eggs or clutches. Dormancy. Predators and shredders.
Group "d "	*Boyeria sp.* (ODO) *Platycnemis* sp. (ODO) *Coenagrion* sp. (ODO)	*Trithemis* sp. (ODO) *Ceriagrion* sp. (ODO) *Chalcolestes* sp. (COL)	Medium-large insects. Semivoltine with long life cycles. Different reproduction techniques. Fliers, thus aerial dispersal. Diapause, crawlers, feeding on other macroinvertebrates.
Group "e"	Culicinae (DIP) Ephydridae (DIP) Eriopterini (DIP) Chironomidae (DIP) Simuliinae (DIP) *Ecnomus* sp. (TRI) *Glossosoma* sp. (TRI) *Cheumatopsyche* sp. (TRI) *Polycentropus* sp. (TRI)	*Agraylea* sp. (TRI) *Potamanthus* sp.(TRI) *Micronecta* sp. (HET) *Baetis* sp. (EPH) *Cloeon* sp. (EPH) *Ferrissia* sp. (MOL) *Stagnicola* sp. (MOL) *Physa* sp. (MOL) *Planorbis* sp. (MOL) *Theodoxus* sp. (MOL) *Proasellus* sp. (CRU)	Small-medium size. Short life-cycle, uni or plurivoltines. Cemented eggs. Aquatic respiration. Crawlers temporarily attached. Shredder or scrapers or other habits.
Group "f"	*Ecdyonurus* sp. (EPH) *Caenis sp.* (EPH)		Medium sized, monovoltine crawlers and shredders. Cemented eggs and cocoons. Aquatic respiration.
Group "g"	*Glaenocorixa* sp. (HET) *Parasigara* sp. (HET) *Corixa* sp. (HET) *Cymattia* sp. (HET) *Microvelia* sp. (HET) *Gerris* sp. (HET)	*Mesovelia* sp. (HET) *Dytiscus* sp. (COL) *Ilibius* sp. (COL) *Gyrinus* sp. (COL) *Noterus* sp. (COL)	Medium-small swimmers or crawlers. Shredders and piercers, feeding on macroinvertebrates. Aerial respiration, cemented clutches. Different life cycle duration and reproductive cycles per year.
Group "h"	Nais sp. (OLI)		Different size, multivoltine, burrowers interstitial, deposit feeders feeding on detritus or microorganisms. Cocoons. Tegument respiration, aquatic drift.

Data extracted from Usseglio-Polatera *et al.* (2000a). No representative species of group "a" (Porifera and Bryozoa) were found. CRU, Crustacea; HIS, Hirudinea; MOL, Mollusca; BIV, Bivalvia; OLI, Oligochaeta; DIP, Diptera; EPH, Ephemeroptera; TRI, Trichoptera; ODO, Odonata; COL, Coleoptera; HET, Heteroptera.

After determining functional groups, we computed functional groups richness (FG richness, number of functional groups) and functional groups diversity, using the Simpson index (FG diversity = $1/\Sigma p_i^2$; where p_i was the proportion of individuals belonging to each functional group at a given site).

Lateral environmental gradients

Three main lateral environmental gradients have been described as affecting taxonomic structure of macroinvertebrates in the Middle Ebro floodplain (Gallardo *et al.* 2008), namely 1) hydrological disturbance, 2) confinement and 3) agricultural pressure. Based on the assumption that the functional structure at a local scale is driven by the same environmental factors than the taxonomic structure (Heino *et al.* 2007) the present study focuses on the response of the invertebrate traits to these three gradients.

Hydrological Disturbance— In this paper, hydrological disturbance in each site and date was obtained by measuring three parameters that accounted for hydrological spatial and temporal variability: flood magnitude, flood duration and flood frequency (Table 12). These are defined as follows:

- Flood magnitude (FM) – water-column depth relative to the maximum and minimum water-column depths in the study period, calculated as

  $$\text{FM}\ (\%) = (\text{WL}-\text{WL}_{min})100/\text{WL}_{max},$$

  where WL is the water-level of a site, and WL_{min} and WL_{max} are the minimum and maximum water-levels, respectively, in the studied period. Water column depth was measured using pressure-based meters with 1-cm resolution (DI-241 Diver, Van Essen Instruments$^{®}$) installed in each site.

- Flood duration (FD) – number of days that the river flow rose above the established connection limit in a 365-day period before each sampling date in each site. For example, flood limit in NOL1 is 400 $m^3\ s^{-1}$. During one year (from 8 March 2005 to 8 March 2006) the river flow rose above 400 $m^3\ s^{-1}$ on 36 days. Thus, this is the FD value for NOL1 in 8 March 2006. The river flow was extracted from public available data (Spanish Water Authority, http: //www.chebro.es). Flood limits were calculated from a digitalized elevation model (DEM) and validated in the field.

- Flood Frequency (FF) –considered as the number of flood events when the river flow raised above the established connection thresholds in a 365-day period before each sampling date in each site. Flood frequency differs from flood duration in that we considered the number of flood events independent of their duration. In the example above, nine flood events were recorded during one year after the sampling date (from 8 March 2005 to 8 March 2006). Thus, 9 is the FF value for NOL1 in 8 March 2006.

No differences in these parameters were expected among the three sampling stations established in each NOL, thus the same value of hydrological disturbance was assigned to them.

Confinement— The reduction in water turnover in wetlands that had reduced seepage, either because of river embankment or sediment accumulation, was considered here as "confinement". During confinement conditions, dissolved salts and organic nutrients from groundwater seepage, runoff and autogenic processes accumulate, enhancing primary productivity (Tockner *et al.* 1999a). Therefore, an increase in the concentration of dissolved solids, chlorophyll-*a* and dissolved organic nitrogen has been used to identify confinement situations (Table 12) as follows:

- Total Dissolved Salts (TDS) – determined from filtered water samples according to standard methods (APHA 1989).
- Chlorophyll a (Chl.a) – Phytoplankton photosynthetic pigments were analyzed from water samples using the Spectrophotometric Method (APHA 1989).
- Dissolved Organic Nitrogen (DON) – assessed in filtered water samples with a continuous flow analyzer (FLOWSYS-SYSTEA®).

Agricultural pressure— Agricultural seepage was a source of inorganic nutrients, mostly nitrate (NO_3^-) that accumulates in NOLs (Gallardo *et al.* 2008). Phosphate, which is usually related to agricultural impact, was not included here because it was always below the detection limit. Therefore, nitrate was used as an indicator of agricultural pressure (Table 12).

- Nitrate (NO_3^-) – determined from filtered water samples analysed by ion chromatography (FLOWSYS-SYSTEA®).

Table 12 Mean (SD) values of hydrological and environmental descriptors in the Ebro River channel (RS) and three oxbow lakes (NOL1-3). Variables in bold were significantly related to biological traits by multivariate analysis.

Variable	**Abbreviation (units)**	**RS n= 6**	**NOL1 n= 18**	**NOL2 n= 18**	**NOL3 n= 18**
Flood Duration	FD (nºdays yr^{-1})	88.33 (13.46)	31.82 (4.91)	2.40 (1.24)	0.83 (0.38)
Flood Frequency	FF (nº)	8.33 (1.37)	8.18 (0.73)	0.80 (0.41)	0.83 (0.38)
Flood Magnitude	FM (%)	6.41 (5.96)	11.67 (9.01)	6.86 (4.43)	17.82 (6.81)
Total Dissolved Solids	TDS (mg L^{-1})	1002 (304)	1779 (952)	1824 (459)	1929 (759)
Chlorophyll a	Chl.a (µg L^{-1})	18.78 (18.09)	14.40 (17.21)	6.81 (10.03)	33.69 (26.29)
Dissolved Organic Nitrogen	DON (mg L^{-1})	0.36 (0.44)	0.11 (0.20)	0.12 (0.45)	0.29 (0.27)
Nitrate	NO_3^- (mg L^{-1})	16.00 (4.60)	1.82 (3.11)	42.94 (6.97)	1.42 (3.12)

Data Analyses

A non-parametric analysis of variance (Kruskal-Wallis test, $\alpha = 0.05$) was used to study differences among sites aligned along a gradient of lateral hydrological connectivity. Trait composition (abundance of each of seven functional groups), richness (FG richness) and diversity (FG diversity) were response variables, and the positions along the gradient of hydrological connectivity (RS, NOL1, NOL2 and NOL3) were different levels of the independent variable (factor).

Multivariate ordination techniques were used to analyze the relationships among functional groups of invertebrates and environmental variables serving as surrogates for hydrological disturbance (FF, FD and FM), confinement (TDS, DON and Chl.a) and agricultural pressure (NO_3^-). Redundancy Analysis (RDA) was used because the length of the species gradient, assessed by Detrended Corresponded Analysis, was short (less than 2) and thus a linear relationship between species and environmental variables was expected (Legendre and Legendre 1998). Environmental variables were previously log (X+1) transformed and checked to be un-correlated (Spearman correlation ratios < 0.6). To avoid multi-collinearity problems we included in the RDA only those variables selected as significant by stepwise forward selection ($\alpha = 0.05$) and with inflation factors below 10 (ter Braak and Šmilauer 2002). Significance of the RDA model was assessed through permutation tests of the full model and by first axis (Legendre & Legendre 1998).

All statistical analyses were run using R (version 2.5.1, R Development Core Team, 2007); functions used can be consulted in Appendix 4.

Results

Trait differences along the lateral hydrological connectivity

We collected a total of 50,233 individuals belonging to 54 genera classified into seven functional groups. Taxonomic richness in functional groups varied from 19 taxa in group “e” to only one in group “h” (Table 13).

A decrease in total abundance coinciding with a decrease in surface connection established by the gradient of lateral hydrological connectivity was observed. Moreover, non-parametric Kruskal-Wallis tests demonstrated that sites differed significantly in their invertebrate trait composition, richness and diversity (Fig. 18 and Table 13). Only the abundance of Groups “c” and “g” showed no significant differences among sites.

Six out of seven functional groups were present at the river site (RS). Among them, Groups “h” and “e” clearly dominated (Fig. 18). Functional groups inhabiting the RS included insects belonging to Diptera, Trichoptera, Heteroptera and Coleoptera families, and also aquatic oligochaetes. Group “d”, composed of predatory dragonflies, was the only group not present in RS. Group “e” was dominant in NOL1 and NOL3, and Group “b”, which includes most of the non-insect taxa, dominated NOL2 (the NOL located at an intermediate position along the connectivity gradient) (Fig. 18).

Table 13 Mean (SD) values of functional groups abundance, richness and diversity in the Ebro river channel (RS) and three oxbow lakes (NOL1-3).

Variable	Units	RS	NOL1	NOL2	NOL3	Kruskal-Wallis		
		n= 6	n= 18	n= 18	n= 18	χ^2	d.f.	*p*
Group “b”	(CPUE)	4.00 (4.19)	4.24 (7.80)	53.53 (128.7)	8.06 (32.94)	23.35	3	<0.001
Group “c”	(CPUE)	3.33 (5.82)	1.12 (2.69)	0.33 (0.82)	0.44 (1.04)	5.62	3	0.13
Group “d”	(CPUE)	0.00 (0.00)	6.94 (19.65)	0.13 (0.35)	0.67 (1.28)	8.20	3	0.04
Group “e”	(CPUE)	513.2 (502.4)	253.3 (567.3)	19.30 (19.92)	28.05 (33.02)	20.02	3	<0.001
Group “f”	(CPUE)	8.00 (8.65)	37.24 (94.85)	0.07 (0.26)	0.33 (0.97)	21.09	3	<0.001
Group “g”	(CPUE)	21.50 (52.18)	0.06 (0.24)	0.07 (0.26)	0.11 (0.47)	5.07	3	0.17
Group “h”	(CPUE)	4031 (4559)	31.65 (33.74)	13.67 (17.29)	14.56 (26.21)	22.30	3	<0.001
N	(CPUE)	4581 (4510)	334.5 (593.6)	87.10 (138.84)	52.21 (71.58)	23.99	3	<0.001
FG richness	Nº groups	4.67 (0.51)	3.94 (1.20)	3.33 (0.49)	2.78 (1.06)	17.62	3	0.001
FG diversity		1.25 (0.18)	1.82 (0.63)	2.12 (0.51)	1.73 (0.54)	10.20	3	0.02

Results of non-parametric analysis of variance between each biological descriptor each and the position along the gradient are shown. CPUE, catches per unit effort; N, total invertebrate abundance; FG richness, number of functional groups; FG diversity, Simpson diversity of functional groups; *p* = significance value; χ^2, Chi-squared distribution, d.f., degrees of freedom.

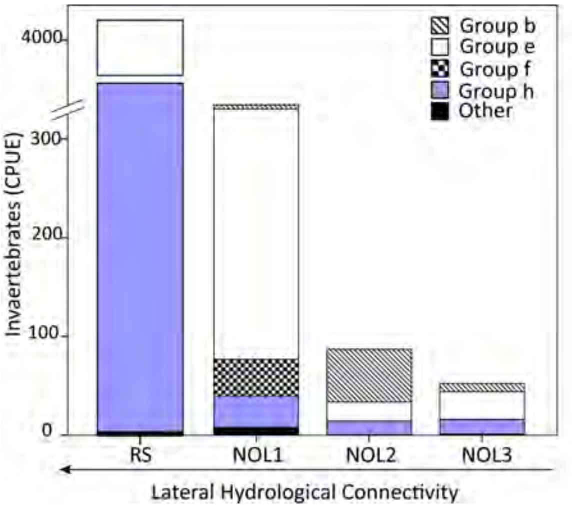

Figure 19 Functional groups composition of the Ebro River (RS) and three oxbow lakes (NOL1-3). Characteristics and taxonomic composition of functional groups are summarized in Table 11. CPUE, catches per unit effort (see description in text). “Other” includes Groups “c”, “d” and “g” which accounted for less than 5% of the total macroinvertebrate abundance.

Functional groups richness varied significantly along the lateral hydrological connectivity, being highest in RS and decreasing in NOLs from NOL1 to NOL3 (Fig. 19). Functional groups diversity also varied significantly across the connectivity gradient, but showed a different pattern, peaking in NOL2 (intermediate wetland in the connectivity gradient), where functional groups diversity was two times higher than in the RS (Fig. 19).

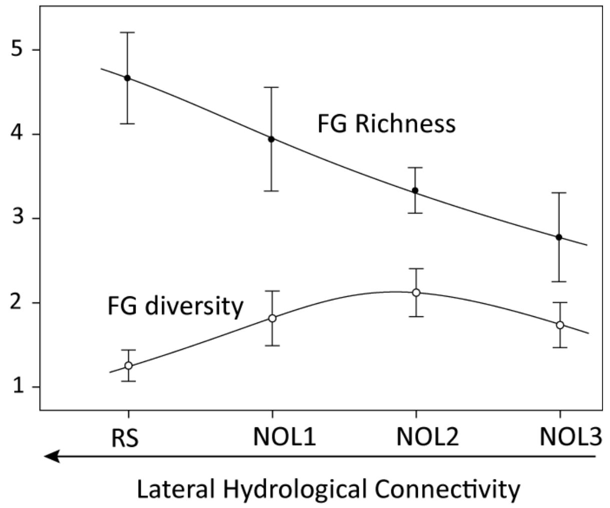

Figure 19 Functional groups richness and functional groups diversity in the Ebro River (RS) and three natural oxbow lakes (NOL1-3).

Relationship between lateral gradients and invertebrate biological traits

Significant variables extracted by forward selection included nitrate, dissolved solids, flood duration and frequency (NO_3^-, TDS, FD and FF). RDA performed on abundance of seven functional groups and these four selected variables was highly significant (Permutation Test on the full model, $p < 0.005$); with the first two axes explaining 43% of invertebrate variability (Permutation Test by axis, $p < 0.005$). At least one variable of each lateral gradient considered was selected (Table 14).

Table 14 Results of redundancy analysis performed using four environmental variables (explanatory variables) and abundance of seven functional groups of macroinvertebrates (response variables).

	RDA1	**RDA2**
Nitrate concentration	0.04	-0.93
Total dissolved solids concentration	-0.28	0.31
Flood duration	0.83	0.23
Flood frequency	0.70	0.44
Accumulated variability accounted by each RDA-axis	**33%**	**43%**

The first RDA axis was related to flood duration and frequency (FD and FF), which were surrogates for hydrological disturbance. Hydrological disturbance was highest in RS and decreased in NOLs from NOL1 to NOL3 (Fig. 20).

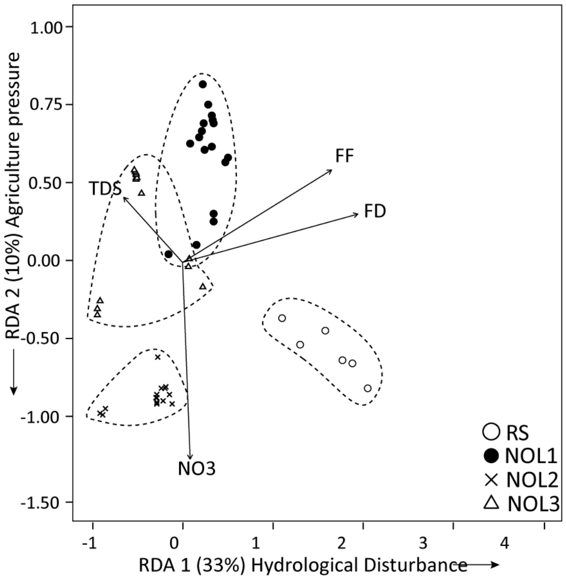

Figure 20 Results of Redundancy Analysis performed between abundance of seven functional groups and four environmental variables. Variables were previously selected by forward selection (α = 0.05). TDS: total dissolved solids concentration, NO3: nitrate concentration, FF: flood frequency, FD: flood duration, RS: River site, NOL: natural oxbow lake.

The second RDA axis was negatively related to nitrate concentration (NO_3^-), a surrogate of agricultural pressure. NOL2, where agricultural pressure was more intense, showed the highest concentrations of NO_3^- (up to 50 mg L^{-1}). Nitrate concentration was also high in RS (up to 20 mg L^{-1}) because of extensive agricultural land cover on the watershed as a whole. In contrast, nitrate concentration was low in NOL1 and NOL3 (< 5 mg L^{-1}). Wetlands were arranged in a longitudinal hydrological gradient in second RDA axis, from NOL1 situated upstream to NOL3, NOL2 and RS sites situated progressively downstream.

Total dissolved salts (TDS), the surrogate of confinement, was weakly related to both RDA1 (hydrological disturbance) and RDA2 (agricultural pressure) (Table 16). Highly confined sites, such as NOL3, were isolated from both the river pulses and sewage coming from agriculture fields; NOL2 and NOL1 were less confined because of higher water-table variability.

Functional traits as bio-indicators of environmental gradients

To identify potential bio-indicators of hydrological disturbance and agricultural pressure, we projected the optima (maximum density) and distribution of functional groups onto the first and second RDA-axis, respectively (Fig. 21). We found that Group “g” was related to both hydrological and agricultural disturbance. This group included aquatic bugs and coleopters that were small in size or exhibited multivoltinism. Beyond these, aquatic worms included in Group “h” peaked at highly hydrologically disturbed sites (i.e., those highly influenced by the river), while crustaceans and bivalves of Group “b” peaked at sites affected by agricultural sewage.

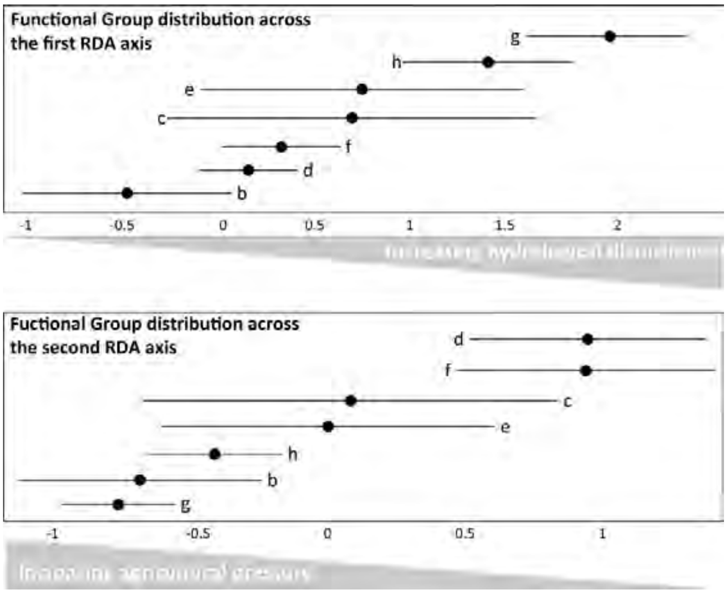

Figure 21 Functional groups distribution across main environmental gradients identified after Redundancy Analysis in the Middle Ebro Floodplain. Mean groups distribution (optima) is represented by black dots; black lines represent the standard deviation of the groups’ distribution.

Discussion

Trait differences along the lateral hydrological connectivity

High spatial heterogeneity and diverse food resources enhanced richness of functional groups in the river channel, but only certain combinations of traits dominated the river community given its particular environmental conditions (e.g., Oligochaeta in Group “h” and Chironomidae in Group “e”, which dominated the river channel). As reported by several authors, disturbance frequency in the river channel selects for those traits that allow organisms to adapt to a changing environment and to recuperate faster after disturbance, such as short life cycles, asexual reproduction, multiple cycles per year, substrate-attachment forms, and crawling or burrowing locomotion (Townsend and Hilldrew 1994, Townsend *et al.* 1997a, Townsend *et al.* 1997b, Usseglio-Polatera *et al.* 2000a). Based on the work of these authors, we should have expected to find that species in the river channel were highly specialized in their feeding behavior, typically filter feeders and scrapers that fed on suspended particles and algae. In contrast, we found a river community that was highly diversified with respect to their feeding behavior, and included predators, shredders, piercers, scrapers and deposit feeders. According to Scarsbrook and Townsend (1993), this situation could reflect a combination of low disturbance frequency and high habitat heterogeneity, and thus high refuge and resource availability that would allow species with different feeding behavior to coexist.

As we move from the river channel to disconnected wetlands within the floodplain, we expect to find that the influence of hydrological disturbance decreases while biotic interactions become increasingly important (Wellborn *et al.* 1996, Poff 1997). Consequently, hydrological stability in floodplain wetlands should allow organisms to reach large sizes and attain long life spans, whereas closer biotic interaction would force them to diversify their feeding behaviour, reproduction and locomotion techniques (Townsend and Hilldrew 1994, Townsend *et al.* 1997a, Townsend *et al.* 1997b, Usseglio-Polatera *et al.* 2000a). In accordance with these ideas, we found that crustaceans, bivalves and some insects (e.g., Odonata and Coleoptera larvae which used active aerial or aquatic dispersal) were representative taxonomic inhabitants of the disconnected floodplain wetlands. They were mostly large-sized shredders, filterers and piercers that fed on the wide variety of detritus and macrophytes available, and also predators that fed on other invertebrates.

It is worth highlighting, however, that several functional groups showing adaptation to disturbance (e.g., small size, attachment to substrate, multivoltinism, cemented eggs or clutches, filter-feeding, crawling and burrowing) in Groups “e” and “g” were present in both the river channel and floodplain wetlands, but with differing degrees of dominance. This suggests that organisms dominating the river channel were transported during floods to floodplain wetlands, where they found refuge but did not reach high densities. Generalist species that could have benefited from river pulses to colonize new habitats included *Ecnomus* sp., *Micronecta* sp., and *Nais* sp. In contrast, specialist species present in the studied floodplain wetlands that did not appear in the river channel included the predatory dragonflies, *Boyeria* sp., *Trithemis* sp. and *Coenagrion* sp. Consistent with our expectations and in accordance with the Intermediate Disturbance Hypothesis (Connell 1978), the diversity of functional groups was highest at floodplain wetlands exhibiting an intermediate level of disturbance, where both types of species coexisted (i.e. generalist and specialist).

It is worth noting that important habitats, such as secondary channels, backwaters, temporary groundwater-fed pools and hydrologically inter-connected water-bodies were absent in our study area. However, the selected floodplain wetlands represent a unique type of ecosystem (oxbow lake) and cover a range of spatial and temporal hydrological connectivity characteristic of floodplains of large rivers worldwide. Moreover, despite the short lateral hydrological gradient studied here, we found a significant response of macroinvertebrate trait composition, richness and diversity. Such findings underscore the importance of every connectivity type (longitudinal and lateral) in the structure and functionality of the whole river floodplain system (Amoros and Bornette 2002).

Relationships between lateral gradients and invertebrate biological traits

The classification of organisms into functional groups was beneficial for the analysis of the functional adaptation of invertebrates to environmental gradients. As such, it was helpful in disentangling the response of several biological traits to lateral environmental and hydrological gradients (Usseglio-Polatera *et al.* 2000a) that significantly affected invertebrate functionality.

Previous studies analyzing taxonomic patterns have identified limitations in discriminating pressure effects, for example between hydrological, physic-chemical and trophic gradients (Gallardo *et al.* 2008). These difficulties reflect the fact that hydrological connectivity involves multiple patterns and processes, including the superficial transport of suspended solids, seepage input of dissolved solids, accumulation of organic matter during confinement and export of organic matter during large, erosive floods (Tockner *et al.* 1999a). An analysis of these processes can be confounded by confinement-related effects and agricultural pressures, which also involve changes in salts and nutrients. In contrast, the present study found that functional traits were useful in discriminating environmental gradients, showing that increasing disturbance, either hydrological or agricultural, led to domination by Group “g” including organisms with small size, multiple cycles per year and asexual reproduction. The presence of these functional traits reflected the adaptation of communities to frequent and intense disturbance, as suggested by other authors (Dolédec *et al.* 2006, Mellado *et al.* 2008). Furthermore, agricultural pressure and hydrological disturbance could be discriminated from one another on the basis of the abundance of Group “b” and Group “h”, respectively.

Beyond hydrology and water chemistry, the remaining variability in invertebrate traits could be related to several unmeasured variables, such as habitat characteristics (e.g., vegetation cover and substrate size) and biotic interaction (e.g., fish predation and competition with other species) (Batzer and Wissinger 1996, Wellborn *et al.* 1996). Nevertheless, the studied gradients explained almost 50% of the existing variability in the trait structure, and each of the gradients has been shown to be important in explaining the trait structure.

The functional classification of macroinvertebrates: a tool for identifying bio-indicators

Given the large number of biological and ecological traits that could be related to environmental gradients – there are more than 50 biological and 60 ecological trait categories in the bibliography – the classification of organisms into a limited number of homogeneous groups strongly simplifies their use. Invertebrates included in each functional group are expected to have similar life-histories and behavior, and thus show the same response to main environmental gradients (Statzner 2001). However, our results showed that some functional groups have wide distributions (e.g., Group “c”;

Fig. 21), so we should take these indicators with caution. A point to note is that the species in each group are not the same as those in the original classification of Usseglio-Polatera *et al.* (2000a), but only include those present in our study area. Therefore, more studies are needed to better assess the ability of each functional group to indicate environmental gradients or human impacts. Another point to note is that a complex combination of traits was represented by each functional group, and thus the direct relationships between particular traits (e.g., size, feeding habitats) and environmental gradients were difficult to identify. To achieve a better understanding of how spatial and environmental gradients act as a template for macroinvertebrate structure and functionality in complex floodplain ecosystems, future investigations should evaluate the separate effects of lateral gradients on particular traits. Besides the difficulty of working with a high number of traits, testing the simultaneous relationship of taxa abundance, particular traits and environmental factors suppose another challenge that is yet to be statistically solved and that is known as the “fourth-corner problem” (Legendre *et al.* 1997).

Finally, it is worth highlighting the fact that, despite their key role in river-floodplain processes and ecosystem biodiversity (Amoros and Roux 1988, Ward and Stanford 1995, Tockner *et al.* 1999b, Amoros and Bornette 2002), floodplain habitats considered as those disconnected wetlands located in the floodplain, have been systematically ignored in ecological assessments of large rivers in Europe. We believe that future studies should reinforce the potential of the functional grouping of invertebrates to assess ecological integrity in river-floodplain habitats considered as a whole.

Chapter 4

Testing the response of the macroinvertebrate functionality to flooding and confinement[4]

Abstract

The aim of the present study was to investigate the relative importance of flooding- and confinement-related environmental features in explaining macroinvertebrate trait structure and diversity in a pool of wetlands located in a Mediterranean river floodplain. To test hypothesized trait-environment relationships, we employed a recently implemented statistical procedure, the fourth-corner method. We found that flooding-related variables, mainly pH and turbidity, were related to traits that confer an ability of the organism to resist flooding (e.g., small body-shape, protection of eggs) or recuperate faster after flooding (e.g., short life-span, asexual reproduction). In contrast, confinement-related variables, mainly temperature and organic matter, enhanced traits that allow organisms to interact and compete with other organisms (e.g., large size, sexual reproduction) and to efficiently use habitat and resources (e.g., diverse locomotion and feeding strategies). These results are in agreement with predictions made under the River Habitat Templet for lotic ecosystems, and demonstrate the ability of the fourth-corner method to test hypothesis that posit trait-environment relationships. Trait diversity was slightly higher in flooded than in confined sites, whereas trait richness was not significantly different. This suggests that although trait structure may change in response to the main environmental factors, as evidenced by the fourth-corner method, the number of life-history strategies needed to persist in the face of such constraints remains more or less constant; only their relative dominance differs.

Introduction

In recent years, a functional approach to ecosystem analysis based on multiple biological traits of species has provided new insights into the study of aquatic assemblages and their adaptation to environmental constraints (Statzner 2001, Bonada *et al.* 2006). This functional approach is ecologically meaningful because it takes into account several invertebrate characteristics that can be directly related to ecosystem structure and functionality (Statzner 2001, Statzner *et al.* 2001). For instance, body shape and attachment to substrate have been related to the ability of invertebrates to resist flooding (Townsend & Hilldrew 1994); aerial respiration has been related to frequent episodes of oxygen depletion (Dolédec *et al.* 2006); locomotion and substrate relationship have been related to habitat characteristics (Heino 2005); and reproductive strategies have long been related to

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ecosystem stability (Townsend & Hilldrew 1994). The functional approach is also broadly applicable, providing a useful tool for predicting changes in aquatic structure driven by environmental factors, such as flooding (Townsend *et al.* 1997), land use (Dolédec *et al.* 2006), water pollution (Charvet *et al.* 2000, Gayraud *et al.* 2003, Dolédec & Statzner 2007), invasive species (Statzner *et al.* 2008) and climate change (Bonada *et al.* 2007).

However, certain problems arise in studying relationships among trait affinity, taxa abundance and environmental constraints; in particular, how to relate these three aspects simultaneously and how to test the significance of their relationships, a difficulty known as the “fourth-corner problem” (Legendre *et al.* 1997). A range of trait-based analysis strategies have been used, including clustering species with similar combinations of traits (Usseglio-Polatera *et al.* 2000a), analyzing the relationship between patterns in species traits and habitat use using multivariate analytical techniques (Dolédec *et al.* 1996) and weighting the trait affinity of species by their abundance using regression models (Charvet *et al.* 2000). More recently, Dray and Legendre (2008) provided an improved statistical method to address this issue, namely the fourth-corner statistic. This improved procedure, based on the original method developed by Legendre *et al.* (1997) offers the opportunity to work with species abundance or presence/absence, and several testing procedures for confirming or rejecting hypotheses positing trait-environment relationships.

Floodplain aquatic environments offer an ideal system for testing such hypotheses because several different environmental situations can be found along the lateral connectivity gradient (Amoros & Bornette 2002). Moreover, macroinvertebrates are an especially suitable model organism for assessing environmental changes because of their multiple forms, behaviors and habitats used (Rosenberg & Resh 1993). In this sense, a template for river ecosystems (River Habitat Templet, Townsend & Hilldrew 1994) enables predictions to be made about the traits of species that are more likely to occur under particular conditions. These predictions have been tested for a broad range of aquatic organisms in floodplain habitats, mostly located in the Rhône River (Dolédec & Statzner 1994, Resh *et al.* 1994). However, less information is available from other river floodplains, particularly from Mediterranean ecosystems, where floodplain aquatic environments are controlled by changes in habitat structure, salinity and nutrient concentration that are mainly related to floods and droughts, and indirectly to river regulation and agricultural practices (Gasith & Resh 1999).

Many researchers have stressed the importance of environmental features, such as habitat structure, water chemistry and trophic conditions, in determining the trait-structure of freshwater macroinvertebrate assemblages in both lentic (Wellborn *et al.* 1996, Heino 2005, 2008) and lotic habitats (Batzer & Wissinger 1996, Dolédec *et al.* 2006, Piscart *et al.* 2006, Mellado *et al.* 2008). These studies have often documented direct effects of environmental features on the selection of particular life-history strategies, and indirect effects of environmental factors on the abundance and diversity of algae and macrophytes; this latter relationship, in turn, controls the amount of substrates and food resources available for invertebrates (Woodward & Hilldrew 2002). In this context, previous studies have reported adaptations to local environmental conditions, biological interaction and predation along a hydrological connectivity gradient (Dolédec & Statzner 1994, Resh *et al.* 1994, Mellado *et al.* 2008). Therefore, we would expect environmental features that reflect flooding and confinement conditions, as representatives of the two extremes of the hydrological connectivity gradient, to limit the range of life-history strategies of species that inhabit these particular conditions.

It has been suggested that functional biodiversity in frequently flooded sites (i.e., those submitted to extreme environmental constraints) decreases, while that in confined sites (i.e., those reporting highest biotic interaction) increases (Statzner *et al.* 2004, Mouillot *et al.* 2006). This suggestion is based on the assumption that unfavorable conditions limit the range of life-history strategies capable of supporting survival, while biotic interaction enhances species competition and trait diversification (Statzner *et al.* 2004). However, hypotheses that posit differences in functional diversity between flooded and confined sites remain to be tested in floodplain habitats. Because functional diversity, rather than species diversity, is the key driver of important ecosystem processes such as productivity, stability and recovery (Mouillot *et al.* 2006), a greater understanding of functional diversity should contribute greatly to our knowledge of floodplain functionality and aid in ecosystem management.

To date, the improved fourth-corner methodology has not been used to assess hypotheses concerning trait-environment relationships in floodplain habitats. In the present study, we use this statistical procedure to investigate the relative importance of flooding- and confinement-related environmental features in explaining the macroinvertebrate trait structure in a pool of wetlands located in a Mediterranean river floodplain (Ebro River, NE Spain). Here, we first describe the environmental features and aquatic communities of the Ebro River and its floodplain. Second, we assess the influence of these environmental factors on trait structure by means of the fourth-corner method. Next, we evaluate differences in trait biodiversity between flooded and confined sites. Finally, we discuss the ability of the fourth-corner method to describe the trait structure.

Material and Methods

Sampling design and procedure

Selected sites for this Chapter included 17 wetlands in the Ebro River and its floodplain along a 100-km length of the river corresponding to the Middle Ebro sector-scale (Fig. 5). Wetlands were divided in two main groups: those that are permanently connected with the river (flooded sites), and those that are only connected during flood pulses (confined sites) (see wetlands characteristics in Table 3).

Flooded sites— Sampling sites within this group are those located in the main river channel (RS), secondary channels (SCs) and backwaters (BWs), all of which are permanently connected to the main river channel at least at one end.

Confined sites— Sampling sites within this group included natural (NOLs) and newly created (COLs) oxbow lakes that do not have a permanent connection with the river channel, and are thus isolated from the river dynamics.

We collected two to three samples in every wetland during two sampling surveys (September 2006 and August 2007). Water and macroinvertebrate samples were taken in each sampling station according to methods described in the General Methodology (Middle Ebro sector-scale).

Macroinvertebrate traits

Macroinvertebrates collected in each sample were analyzed to genus level (except Oligochaeta and Chironomidae), as it has been shown that taxonomic levels higher than species are suitable for studying the functional composition of communities (Dolédec *et al.* 1999, Gayraud *et al.* 2003).

Each trait was composed of 2–8 categories for a total of 60 (e.g., the trait "potential size" was divided into seven categories covering different size ranges). A score was assigned to each genus (ranging from 0= no affinity to 3= high affinity) according to their affinity for each category, which is known as "fuzzy coding" (Chevenet et al. 1994)". Thus, these affinity scores (extracted from Tachet et al. 2000), were assigned to each taxa for the eleven biological traits considered in this study (Table 15).

Table 15 Biological traits and modalities for invertebrates considered in the present study.

Trait	Categories	Trait	Categories
1. Maximum size	< 2.5 mm	8. Resistance form	Eggs, statoblasts, gemmules
	2.5 > size ≤ 5 mm		
	5 > size ≤10 mm		Cocoons
	10 > size ≤ 20 mm		Cells against desiccation
	20 > size ≤ 40 mm		Diapause or dormancy
	40 > size ≤ 80 mm		None
	> 80 mm	9. Locomotion	Flier
2. Respiration	Tegument		Surface swimmer
	Gill		Swimmer
	Plastron		Burrower
	Spiracle (aerial)		Crawler
3. Life cycle	< 1year		Interstitial

duration	> 1 year
4. Potential number of rep. cycles yr^{-1}	< 1
	1
	> 1
5. Aquatic stage	Egg
	Larva
	Nymph
	Adult
6. Reproduction	Ovoviviparity
	Isolated eggs, cemented
	Clutches, cemented or fixed
	Asexual reproduction
	Isolated eggs, free
	Clutches, free
	Eggs or clutches in vegetation
7. Dispersal	Aquatic passive
	Aquatic active
	Aerial passive
	Aerial active
	Temporarily attached
	Permanently attached
10. Food	Deposit feeder
	Fine sediments and microorganisms
	Detritus < 1 mm
	Plant detritus ≥ 1 mm
	Living microphytes
	Living macrophytes
	Dead animal > 1 mm
	Living microinv.
	Living macroinv.
	Vertebrates
11. Feeding habits	Filter-feeder
	Shredder
	Scraper
	Piercer
	Predator
	Parasite

Trait richness was calculated as the number of trait modalities present at a site (Bonada *et al.* 2007). Trait diversity was calculated as the Rao diversity coefficient using the methodology developed by Champely & Chessel (2002) and implemented in package "ade4" (Chessel *et al.* 2004) available in R 2.5.1 statistical software (R Development Core Team 2007). Rao's diversity index allows the diversity in a set of species to be measured using trait dissimilarity between the species, and the distribution of species in sites (Champely & Chessel 2002).

Statistical analyses

First, we used uni- and multivariate analysis of variance (ANOVA and MANOVA) to identify significant differences in environmental characteristics between flooded and confined sites. To reduce the effect of extreme values, variables were previously log(X+1) transformed (except pH).

Second, the existing relationship between transformed environmental variables and species was assessed through a canonical correspondence analysis (CCA, ter Braak 1986). To reduce multicollinearity problems, we ran a manual forward selection, and only variables that had a conditional effect significant at the 10% level ($p \leq 0.1$, Magnan 1994) were selected. In addition, the inflation factor of selected variables was check to be less than 10, ensuring a minimum redundancy among the retained variables (ter Braak and Šmilauer 2002). *P*-values were calculated using the

Monte Carlo Permutation Test (Hope 1968). CCA was performed using CANOCO 4.5 (ter Braak and Šmilauer 2002)

Third, we used the improved fourth-corner methodology to test the relationships between environmental variables and species traits through the link provided by the abundance of species (Dray & Legendre 2008). The null hypothesis H_0 is that species traits are unrelated to the environmental characteristics of the sites, their relationships being mediated by species abundance. To test this hypothesis, we used a combination of permutation tests, as recommended by Dray and Legendre (2008) which reduces Type I errors and increases the power of the link obtained when the three tables are related. To that end, we first permuted site vectors to test the null hypothesis H_1 that the species abundance and the environmental variables are unrelated (permutation model 2, repeated 999 times). After that, we permuted species vectors to test the null hypothesis H_2 that the species abundance and the species traits are unrelated (permutation model 4, repeated 999 times). If both permutation tests were significant, then we can reject H_0 and thus the environmental conditions, species abundance and traits were considered to be effectively linked. In this sense, if α1 is the significant level at which H_1 is rejected, and α_2 is the significant level at which H_2 is rejected, then $\alpha_3 = \alpha_1 \alpha_2$ is the significant level at which H_0 is rejected, thereby $\alpha_1 = \alpha_2 = \sqrt{\alpha_3}$. Additionally, a Bonferroni correction for multiple testing has been also used. Thus, corrected α3 for the twelve environmental variables was use to finally obtain the significance level ($\alpha_3 = 0.05/12= 0.0042$). As a consequence, the significant levels to reject H_1 and H_2 (in permutation models 2 and 4 respectively) was considered to be $\alpha_1 = \alpha_2 = \sqrt{0.0042} = 0.064$. For the fourth-corner analysis, we used function "fourthcorner" included in the package "ade4" (Dray and Dufour 2007) available in R 2.5.1 (R Development Core Team 2007) (Appendix 4). The description of the original technique developed for presence-absence data can be found in Legendre *et al.* (1997) and the extension to abundance data is presented in Dray and Legendre (2008).

Third, we used the non-parametric Wilcoxon Rank sum test to assess differences in trait richness and diversity between flooded and confined sites. We tested the hypothesis that flooded sites have less richness and diversity of traits than confined sites because environmental constraints limit the range of life-history strategies able to cope with flooding disturbance.

Results

General characterization of the study area

Multivariate analysis of variance (MANOVA) showed that flooded and confined sites significantly differed in their environmental characteristics. Turbidity, inorganic nitrogen and organic phosphorus were significantly higher in flooded than confined sites, while the concentrations of dissolved solids, alkalinity, dissolved oxygen, organic matter, organic carbon and chlorophyll-a were higher in the latter (Table 16).

Table 16 Mean and (SD) of environmental variables measured in flooded and confined sites in the Ebro River. Temperature in ºC, all the remaining variables in mg L^{-1}. n= sampling number. Variables in bold showed significant differences between flooded and confined sites (ANOVA, *p*<0.05). Results from MANOVA are shown at the bottom.

Variable (abbreviation)	Flooded (n = 37)	Confined (n = 41)
Turbidity (TSS)	81.10 (70.19)	49.53 (54.99)
Salinity (TDS)	1601.27 (798.64)	1915.89 (849.78)
Alkalinity (HCO3)	204.13 (68.19)	214.21 (124.72)
pH	8.03 (0.36)	7.96 (0.31)
Temperature (T)	20.60 (2.66)	20.56 (3.35)
Dissolved Oxygen (DO)	7.47 (2.19)	7.71 (2.96)
Ash-Free Dry Mass (AFDM)	21.06 (16.42)	33.87 (22.41)
Dissolved Inorganic N (DIN)	4.19 (1.69)	1.55 (2.90)
Dissolved Organic N (DON)	1.24 (1.16)	0.51 (0.23)
Dissolved Organic P (DOP)	27.67 (24.14)	17.93 (52.82)
Dissolved Organic C (DOC)	5.32 (3.26)	7.94 (4.08)
Chlorophyll a (Chl.a)	19.13 (13.18)	20.94 (18.08)
MANOVA Pillai Trace = 0.817; $F_{12,65}$=24.24; *p*<0.001		

The Canonical correspondence analysis (CCA) performed with environmental variables and species abundances retained 9 out of 12 environmental variables and explained 32% of the total variance in species composition (Fig. 22). According to this analysis, flooded wetlands (at the left) were related to the abundance of aquatic worms (e.g., *Nais* sp.), gastropods (e.g., *Stagnicola* sp., *Theoduxus* sp.), crustaceans (e.g., *Atyaephira* sp., *Echinogammarus* sp.) and insects (mostly Trichoptera and Ephemeroptera families). By contrast, confined wetlands (at the right) were related to the abundance of gastropods (e.g., *Physa* sp., *Ferrissia* sp.), crustaceans (e.g., *Procambarus* sp.) and insects (mostly Chironomidae, Odonata, Coleoptera and Diptera families).

Environmental features affecting the macroinvertebrate trait composition

Environmental variables most significantly related to the invertebrate trait structure (i.e., those accounting for a higher number of significant relationships) included pH, turbidity, water organic matter content and temperature. In contrast, organic phosphorus and oxygen were less significantly related to the macroinvertebrate trait structure (Fig. 23).

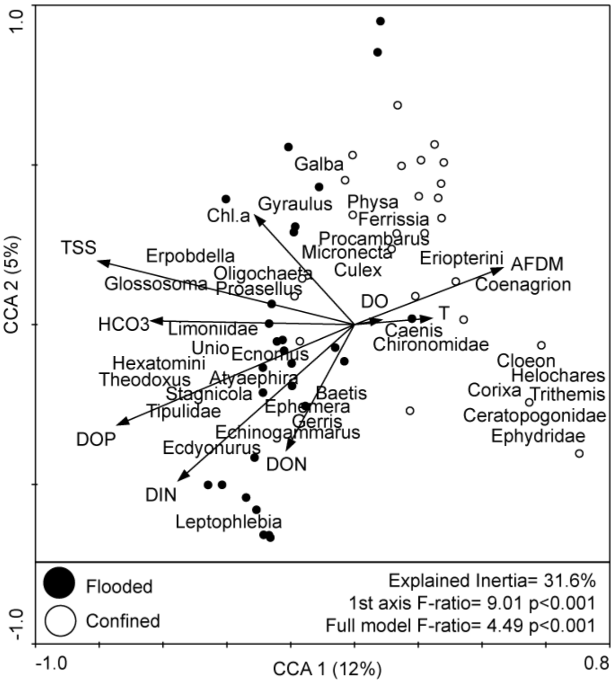

Figure 22 Results of Canonical Correspondence Analysis performed between species abundance and environmental features in the Ebro river-floodplain.

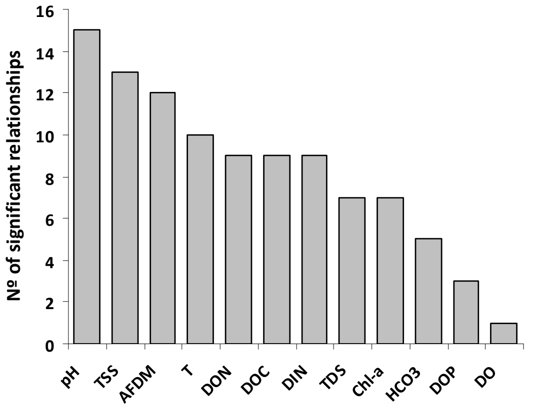

Figure 23 Number of significant relationships found between each environmental factor and biological trait-modalities, obtained by means of the fourth-corner statistic.

On the basis of trait-category responses, environmental variables were divided into two main groups (Tables 17 and 18). First group included pH, turbidity, inorganic nitrogen, chlorophyll a, alkalinity and organic phosphorus that generally had higher values in flooded sites (except HCO3 and Chl-a). Second group included salinity, temperature, oxygenation, water organic matter content, organic nitrogen and phosphorus which generally showed higher values in confined sites (except DON). These two groups of variables showed contrasting relationships with species trait-categories. Hence, trait-categories that showed a general positive relationship with environmental variables in the first group were negatively correlated with environmental variables in the second group, and *vice-versa*

(Tables 17 and 18). A positive relationship indicated that the abundance of individuals showing a particular trait is expected to increase with increasing levels of the environmental factor, whereas a negative relationship indicates the opposite (i.e., a decrease in the abundance of individuals showing a particular trait at increasing values of the environmental variable). Hence, for ease of comparison, we will only describe these positive relationships between each group of variables and trait-categories in general terms.

Among the flooding-related variables in the first group, pH and turbidity showed the highest number of significant relationships with trait categories, whereas organic phosphorus and alkalinity showed the lowest (Table 17). All of the environmental variables in this category were generally positively related to small-sized organisms (2.5–5 mm) that exhibited aerial respiration (by plastron), egg or imago stage, used swimming locomotion and were dispersed by aquatic passive means. These variables were also positively related to organisms with reproductive strategies characterized by the production of cemented eggs that were shredder, scraper and piercers, feeding on coarse plant detritus (>1mm) and living microinvertebrates.

Table 17 Results from fourth-corner analysis performed using species abundance, species functional traits and environmental variables with higher values in flooded sites. The sign (+/-) represents the positive or negative significant Pearson correlation between the environmental variable and the functional trait. For ease of interpretation, only significant relationships (*p*<0.004) are shown (see methodology for further details on statistical models).

TRAIT	CATEGORY	pH	TSS	DIN	Chl.a	HCO3	DOP
1. Maximum size	> 2,5 mm		+	+	+		
	> 20 mm	-				-	
	> 40mm		-				
2. Respiration	Tegument	-					
	Gill		-				
	Plastron		+	+			
4. Nº cycles yr^{-1}	< 1		-				-
5. Aquatic stage	Egg	+				+	
	Nymph	-					
	Imago		+	+			
6. Reproduction	Cemented eggs	+					
	Free clutches	-		-		-	
7. Dispersal	Aquatic passive		+		+		
	Aerial passive	-					
	Aerial active		-		-		
8. Resistance	None	-					
9. Locomotion	Swimmer	+					
	Crawler				-		-
	Burrower	-					
	Temp. attached	-					
10. Food	Detritus < 1 mm	-					
	Plant detritus > 1mm					+	
	Microinvertebrates		+	+			
	Macroinvertebrates		-	-			

11. Feeding habits	Filterer	-					
	Deposit	-					
	Shredder				+		
	Scraper		+		+		
	Piercer		+	+			
	Predator		-	-	-		-
	Parasite	-		-		-	

Among confinement-related variables in the second group, water organic matter content and temperature were most closely related to species traits (Table 18). Environmental variables in this second group were found to favor traits such as large size (>20 mm), aerial passive dispersal and the absence of resistance forms. Environmental variables in the confinement group were related to organisms that produced unprotected (free) clutches or reproduced by ovoviviparity. Finally, the feeding groups associated with the variables in this second group were filterers and parasites, feeding of fine detritus (<1mm).

Table 18 Results from fourth-corner analysis performed using species abundance, species functional traits and environmental variables with higher values in confined sites. The sign (+/-) represents the positive or negative Pearson correlation between the environmental variable and the functional trait. For ease of interpretation, only significant relationships ($p < 0.004$) are shown (see methodology for further details on statistical models).

TRAIT	CATEGORY	AFDM	T	DOC	DON	TDS	DO
1. Maximum size	> 2,5 mm	-	-			-	
	> 20mm	+	+	+			
2. Respiration	Plastron	-	-		-	-	
	Spiracle		-		-		
4. Nº cycles yr^{-1}	=1				+		
5. Aquatic stage	Egg	-	-	-		-	
	Imago					-	
6. Reproduction	Ovoviviparity				+		
	Cemented eggs	-		-	-		
	Free clutches	+	+	+		+	
	Asexual						-
7. Dispersal	Aerial passive	+	+	+			
8. Resistance	None	+		+	+		
9. Locomotion	Swimmer	-					
	Perm. attached			-			
10. Food	Detritus < 1 mm	+	+	+			
	Living macroinv.				-		
11. Feeding habits	Filterer				+		
	Piercer	-	-		-	-	
	Parasite	+	+	+		+	

It is worth noting that many trait categories showed no significant relationship with any environmental variable. These included large potential size (> 80 mm), several reproduction strategies, presence of resistance forms as eggs, cocoons or diapause, and most of locomotion strategies.

Response of trait richness and diversity to flooding and confinement

Trait diversity ranged from 0.82 at NOL1 (a confined oxbow lake) to 28.70 at SC1 (a flooded side channel). Trait richness, in contrast, ranged from 17 traits at NOL3 and COL3 (an oxbow lake and a constructed wetland respectively, both confined) to 44 traits at SC1 and SC3 (both flooded side channels). The non parametric Wilcoxon Rank sum test performed between flooded and confined sites revealed significant differences in trait diversity, which was significantly higher in flooded sites (Wilcox = 582, *p* = 0.08). The differences in trait richness, however, were not significant (Wilcox = 609.5, *p* = 0.13) (Fig. 24).

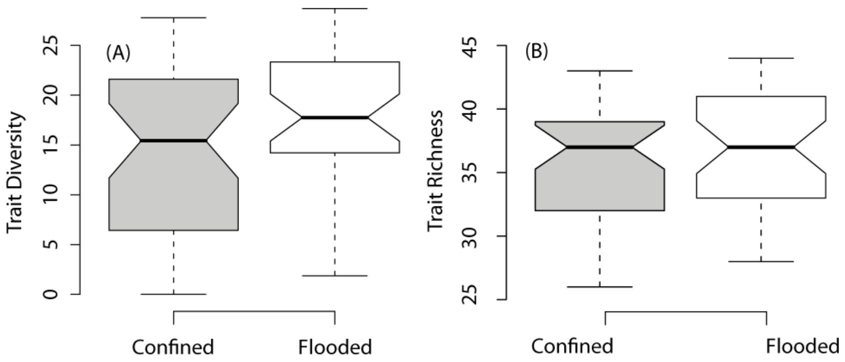

Figure 24 Trait diversity (A) and richness (B) in flooded and confined sites of the Ebro river-floodplain.

Discussion

General characterization of the study area

The river and floodplain wetlands of the Ebro River showed distinctive environmental features with respect to flooded and confined conditions. These differences were reflected in highest turbidity and nutrient concentration in flooded sites, and highest salinity, water organic matter content and organic carbon, oxygenation and primary productivity in confined sites. These results are in agreement with a previous Ebro River basin study conducted on a lower geographical scale (Gallardo *et al.* 2008), as well as with studies performed in other river floodplains (Van den Brink & Van der Velde 1994, Heiler *et al.* 1995, Amoros & Bornette 2002). In flooded wetlands, the effect of hydrological connectivity on environmental features depends on the balance between sediment scouring, turbidity, turbulence and nutrient inputs; the interplay of these factors often results in increased suspended sediments and inorganic nutrients (Heiler *et al.* 1995). In contrast, dissolved salts and organic nutrients introduced by groundwater seepage and runoff, or generated by autogenic processes, tend to accumulate in confined wetlands (Tockner *et al.* 1999), eventually leading to salinization and eutrophication. The high concentration of inorganic nitrogen found in flooded wetlands may also be related to extensive agricultural practices, which are reportedly responsible for 66% of nitrate loads in the Ebro catchment (Torrecilla 2005).

According to multivariate analysis, environmental variables accounted for more than a third of the macroinvertebrate species composition variability. Flooded and confined sites showed distinctive macroinvertebrate composition, in accordance with previous studies performed in the Ebro basin (Gallardo *et al.* 2008) as well as in other floodplains (Gasith and Resh 1999, Reckendorfer *et al.* 2006). In frequently flooded wetlands, invertebrate assemblages has been suggested to be dominated by insect species, particularly from Trichoptera and Ephemeroptera families, as these are considered generalist species capable of resisting survival (Townsend *et al.* 1997a, Usseglio-Polatera *et al.* 2000a, Gallardo *et al.* 2008). The abundance of hirudineans, oligochaetes and chironomids in these sites may be related to the high nutrient concentration of the river. By contrast, crustaceans and insects of Heteroptera, Coleoptera, Odonata and Diptera families became more abundant in confined sites, as these groups are generally supposed to be specialized in habitat and resource exploitation in stable habitats (Townsend *et al.* 1997a, Usseglio-Polatera *et al.* 2000a, Gallardo *et al.* 2008).

Main environmental factors determining the trait composition

It is known that environmental conditions can drive changes in biological traits (Southwood 1988). In this context, we have found evidence that water environmental factors create a template for evaluating the biological traits of organisms that inhabit floodplain wetlands. Based on the response of the trait structure to environmental features obtained using the fourth-corner methodology, we could identify two complementary environmental gradients that structure the functional composition of aquatic invertebrates: flooding and confinement. These gradients are consistent with the characteristics of the study area and describe trait structure in relation to the respective environmental variability of flooded and confined sites.

The first gradient reflects disturbance of wetlands by flooding, since environmental variables in this gradient (e.g., turbidity, inorganic nutrients) are usually related to river water inputs (Heiler *et al.* 1995, Amoros & Bornette 2002). These variables showed a positive relationship with trait categories that confer an ability to (a) resist unfavorable conditions (e.g., small body-shape, protection of eggs), (b) recuperate faster when environmental conditions are less limiting (e.g., short life span, asexual reproduction) (Townsend & Hilldrew 1994), (c) reduce the impact of environmental fluctuations by means of asexual reproduction and the laying of cemented eggs (Townsend & Hilldrew 1994), and (d) effectively colonize new habitats during flooding because of aquatic passive dispersal and swimming locomotion (Mellado *et al.* 2008). Flooding-related variables have also been suggested to influence feeding habits, enhancing shredders, scrapers and piercers that benefit from organic detritus and attached algae (Heino 2008).

The second gradient, related to confinement, exerted a nearly perfect complementary effect on the selection of traits compared to the first. Certainly, variables in the second gradient (e.g., temperature, salinity, organic nutrients) are usually related to stability or confinement in floodplain habitats (Gallardo *et al.* 2009). The second gradient was positively associated with traits that allow organisms to interact and compete with other organisms (e.g., large size, sexual reproduction) and to efficiently use habitat and resources (e.g., diverse locomotion and feeding strategies) (Townsend & Hilldrew 1994, Townsend *et al.* 1997). For instance, large body size, long life span and less than one reproductive cycle per year have commonly been taken as evidence of relatively stable habitats with a low frequency and intensity of flood disturbances (Townsend & Hilldrew 1994, Mellado *et al.* 2008).

Invertebrate trait responses to those two gradients are good examples of the possible extension of the River Habitat Templet (RHT) proposed by Townsend & Hilldrew (1994) to lentic habitats. As originally proposed, this theory predicts traits that are more likely to occur under particular habitat conditions in lotic habitats. Accordingly, the first dimension of the RHT focused on the frequency and magnitude of disturbances, which corresponds to the flooding gradient obtained in the present study. The second dimension of the original RHT focused on the role of refugia in buffering the effects of disturbance; in our study, confinement substitutes for this aspect. The extension of the RHT to the entire river-floodplain habitat has been explored for a broad range of aquatic organisms in the Rhône River (Dolédec & Statzner 1994), where, despite the fact that species traits were significantly related to habitat utilization, the overall results showed little agreement with the RHT predictions (Dolédec & Statzner 1994, Resh *et al.* 1994). Such a mismatch was related to the high spatio-temporal heterogeneity of the floodplain ecosystem and the existence of trade-offs (i.e., different strategies used to face the same constraints) and spin-offs (i.e., correlated traits) between traits (Resh *et al.* 1994). Nevertheless, in the present study, we were able to assess the response of aquatic invertebrates to differences in habitat (flooded vs. confined habitats) mediated by differences in environmental characteristics, such as turbidity, salinity, and inorganic and organic nutrient concentration.

Response of the trait diversity and richness to flooding and confinement

It has been suggested that functional biodiversity decreases with increasing environmental constraints or stress, as surviving species are more likely to be similar to one another (Statzner *et al.* 2004, Mouillot *et al.* 2006). Consistent with these suggestions, we should expect trait richness and diversity to decrease in flooded sites, whereas confined sites should exhibit the opposite response (increased trait richness and diversity). In contrast, we found that trait diversity was slightly higher in flooded sites, whereas trait richness was not significantly different between flooded and confined sites. In this sense, Brinson (1993) reported that less-frequently flooded wetlands are not less functional than frequently flooded ones; the functions are simply different. This suggests that even if trait structure changes in response to the main environmental factors, as evidenced by fourth-corner results, the number of life-history strategies needed to cope with such constraints remains more or less constant; only their relative dominance differs. It thus follows that confined sites may have communities with few, highly dominant trait categories, while in flooded sites the dominance of trait categories may be more equally distributed. These changes in dominance could be explained by the stability of the habitat in confined sites, since it is known that stable conditions may lead to the dominance of few organisms (Connell 1978).

An accurate understanding of environmental factors that affect trait biodiversity may be useful for ecosystem management since increasing trait biodiversity may increase overall organism biodiversity and enhance important ecosystem services, such as detritus processing, nutrient cycling, grazing, predation, leaf litter breakdown and energy transfer (Heino 2005). Thus, further research is needed to identify other features beyond flooding and confinement patterns (e.g., vegetation cover, substrate size) that might contribute to trait biodiversity in the Mediterranean floodplains.

Other factors that may influence trait richness and diversity include spatial heterogeneity (substrate size, macrophyte cover), annual inter-variability or longitudinal connectivity.

The fourth-corner statistic as a tool for describing biological trait composition

Dray and Legendre (2008) provided an improved methodology that differs from other statistical options (e.g., Dolédec *et al.* 1996, Charvet *et al.* 2000, Usseglio-Polatera *et al.* 2000b) in that it seeks to test the significance of the relationship between every trait and environmental constraint. The fourth-corner method, which is based on a previous technique (Legendre *et al.* 1997), also allows relationships among trait affinity, taxa abundance and environmental constraints to be assessed simultaneously, instead of stepwise. Unlike the original method of Legendre *et al.* (1997), it also allows for the use of abundance data.

Although the modified version of the fourth-corner technique is an improvement over the original, some criticisms remain. For example, as previously noted by Dray and Legendre (2008), a multiple interaction form of the fourth-corner method that allows covariates to be introduced has yet to be developed; hence, more complicated hypothesis cannot be satisfactorily tested using this approach.

Also, the fourth-corner method does not take into account potential correlations among traits (trade-offs and spin-offs), although this limitation is also shared by other statistical alternatives. Dray and Legendre (2008) also suggested that is possible to obtain false positives using the fourth-corner method in cases where species abundance is related to species traits or environmental characteristics, but not both. This would lead to the incorrect conclusion that a link exists between traits and environmental characteristics that is mediated by species abundance. Nevertheless, the combined testing approach used in this study is one way to solve this problem and offers a powerful tool for testing trait-environment relationships that can be also applied to other ecological studies involving three data-tables (Dray and Legendre 2008).

Although these various considerations suggest that caveats are in order, the fourth-corner method nonetheless provided evidence to support predictions made under sound ecological frameworks, such as the River Habitat Templet (Townsend & Hilldrew 1994). In addition, it was easily implemented in the R programming language. Ultimately, this research may aid in predicting how communities will change in response to given environmental changes, and thereby provide a guide for biodiversity conservation and biomonitoring programs, and efforts to restore and maintain the quality of stream ecosystems (Rosenberg & Resh 1993, Heino 2005, 2008).

General Discussion

General Discussion

Aquatic community patterns across hydrological and environmental gradients

The interplay between hydrological connectivity, environmental features and human pressure was difficult to disentangle, but in the present study, aquatic communities helped to understand the interaction of these three factors and how they create a template for aquatic organisms. Moreover, the results in the chapters allow us to advance a habitat template adapted for the Middle Ebro river-floodplain, which integrates habitat conditions, macroinvertebrate structure and biodiversity patterns. This template is based on the assumption that habitat conditions, summarized in two axes of spatio-temporal heterogeneity representing hydrological connectivity and environmental variability, select a certain combination of species and biological traits that allow organisms to best survive, reproduce and disperse (Southwood 1988).

(A) Habitat conditions

Hydrological connectivity— The first axis of the Ebro template focuses on the duration of flooding, as it has been identified as the most important characteristic of the flood pulse for aquatic communities (Chapters 2 and 3). Hydrological connectivity integrates a wide range of environmental processes such as water scouring, turbulence, reorganization of habitats, transport of salts, nutrients, seeds and organisms that affect directly and indirectly the presence of aquatic organisms (Amoros and Bornette 2002). Consistently, there is an increasing body of literature dealing with the critical influence of hydrological connectivity in structuring faunal assemblages including macrophytes (e.g., Amoros and Bornette 1999), fish (e.g., Sheaves *et al.* 2007, Roach *et al.* 2009), macroinvertebrates (e.g., Paillex *et al.* 2007, Leigh and Sheldon 2009) and plankton (e.g., Frisch *et al.* 2005, José de Paggi and Paggi 2008).

Hydrological connectivity acted at temporal (floods of different duration) and at spatial (sites in a floodplain submitted to different flood duration) scales, thereby creating a gradient from wetlands showing a close relationship with the river (e.g., secondary channels and backwaters) to those gradually confined (e.g., old confined oxbow lakes and constructed wetlands). This hydrological gradient was found to explain at least one third of the macroinvertebrate species distribution (30%, Chapter 1) and traits composition (35%, Chapter 3), and more than half of the variability observed in total species abundance (65%, Chapter 2) and species richness (70%, Chapter 2) within the Middle Ebro floodplain. Moreover, regarding plankton assemblages (both zooplankton and phytoplankton), the influence of hydrological connectivity was significant but nonetheless slightly lower (responsible of 30-40% of their composition, 40-50% of their abundance and 20-60% of their richness, Chapter 2). The simultaneous study of the richness of zooplankton, phytoplankton and macroinvertebrates indicated that they peak at increasing levels of hydrological connectivity (Chapter 2), thus highlighting the essential role of a hydrologically diverse floodplain in maintaining overall biodiversity (Tockner *et al.* 1999b).

Environmental variability— The second axis of the Ebro template focuses on the variability in the physicochemical habitat, reflected in turbidity, salinity and nutrient concentration, which are important factors for understanding the response of aquatic communities. Certainly, other authors have reported the significant role of the physicochemical habitat on macroinvertebrate species (e.g., Feld and Hering 2007, Wang *et al.* 2007) and trait (e.g., Heino 2008, Mellado *et al.* 2008) composition. In the Ebro floodplain, physicochemical differences across the floodplain were found to explain a low percentage of the macroinvertebrate species (17%, Chapter 1) and trait composition (10%, Chapter 3), possibly because hydrological connectivity already integrates differences in environmental characteristics (Zimmer *et al.* 2000). In fact, environmental factors discriminating flooded (those with high turbidity and inorganic nutrients) and confined (those with high salinity and organic nutrients) wetlands were found to exert a significant influence on the selection of biological traits (Chapter 4). In addition, nutrients affected the abundance and richness of macroinvertebrates (which peaked at 10 mg L^{-1} NO^{-}_{3}) as well as macroinvertebrate diversity and evenness (which dropped at 10 mg L^{-1} NO^{-}_{3}) (Chapter 1). In this sense, there is increasing evidence that nutrients directly affect the productivity and assemblage of primary producers and are indirectly linked to secondary consumers, including macroinvertebrates (Wang *et al.* 2007).

Hydrological connectivity and environmental variability determined a template where predictions about structure and biodiversity patterns under extreme conditions (flooding and confinement) could be made (Fig. 25A). Collectively, hydrological and environmental factors accounted for more than half the variance in the aquatic assemblages. Further studies are nonetheless needed to investigate the role of other potential factors that may account for the unexplained variance, such as land cover (e.g., Strayer *et al.* 2003), submerged and emergent macrophyte cover (e.g., Carpenter and Lodge 1986), substrate structure and composition (e.g., Griffith *et al.* 2001), trophic interaction among groups of organisms (e.g., Blumenshine and Hambright 2003) and pollution derived from human activities (e.g., Woodcock and Huryn 2007).

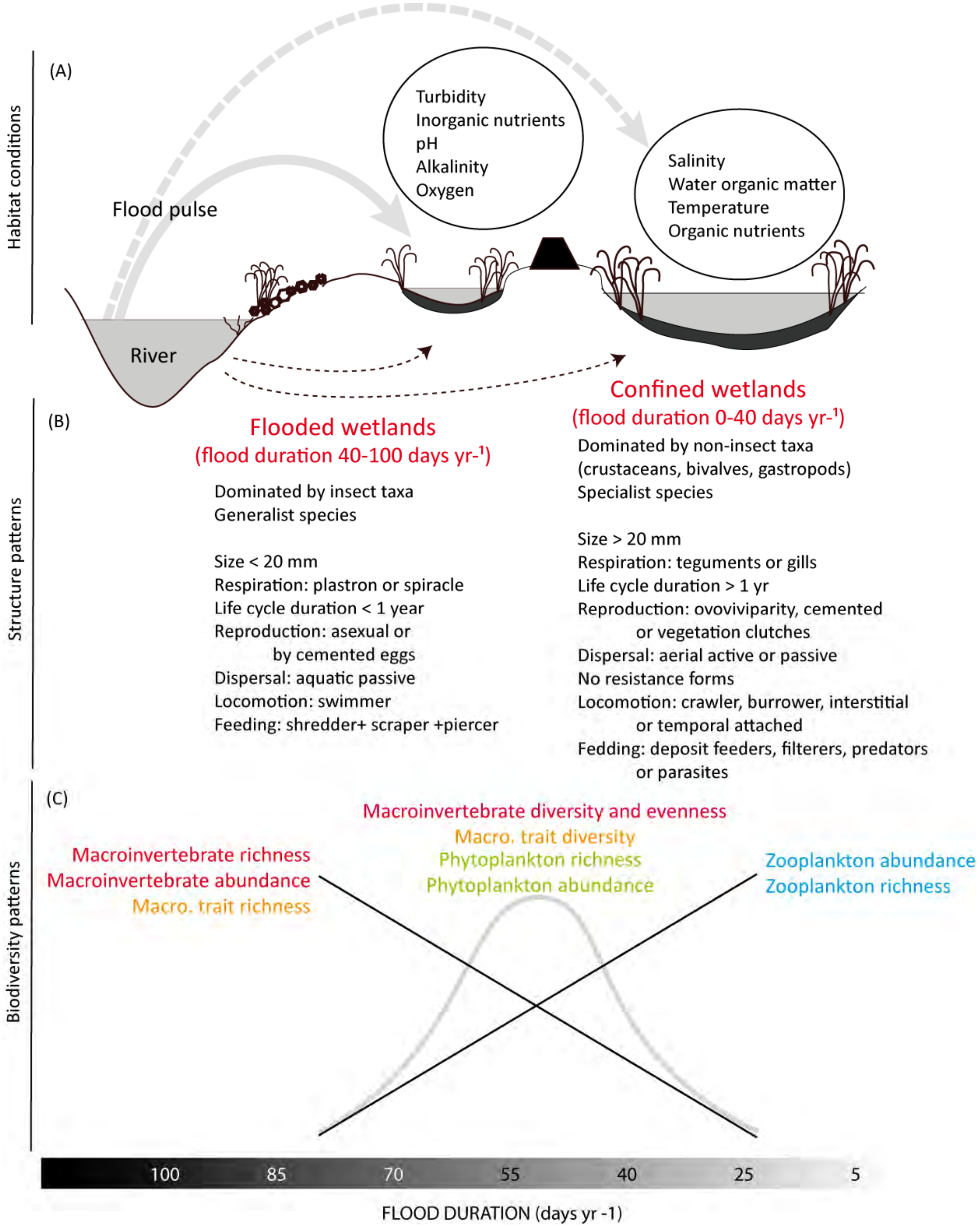

Figure 25 Template of habitat conditions and response of biological structure and biodiversity in the Ebro River, based on results from Chapters 1-4. (A) Habitat conditions related to the frequency and duration of flood pulses. (B) Response of the structure of macroinvertebrates inhabiting flooded and confined wetlands. (C) Response of the biodiversity of aquatic communities to lateral hydrological connectivity. Flood duration has been used as a surrogate of lateral hydrological connectivity.

(B) Macroinvertebrate structure patterns

The response of the macroinvertebrate structure to habitat conditions was consistent with the River Habitat Templet (Townsend & Hilldrew 1994) that predicts which traits are more likely to occur under particular conditions, and supports its extension to lentic habitats.

Invertebrate assemblages in flooded wetlands have been reported to be dominated by insect taxa (Chapter 1). This fact is related to the functional characteristics of insects, as they are often considered generalist species capable of resisting unfavorable conditions, recuperating fast after disturbances and colonizing new habitats (Townsend *et al.* 1997a, Usseglio-Polatera *et al.* 2000). The selected traits identified for confined and flooded sites confirmed this pattern (Fig. 25B). For instance, conditions of high flood duration (implying high turbidity and inorganic nutrients) have been suggested to favor small body-shapes, protection of eggs, short life spans, asexual reproduction, aquatic dispersal and swimming locomotion (Townsend and Hilldrew 1994). Flooding also influences feeding behavior, favoring shredders, scrapers and piercers that benefit from organic detritus and attached algae (Heino 2008).

Non-insect taxa (including crustaceans, bivalves and gastropods) dominate confined wetlands, since these groups generally specialize in habitat and resource exploitation in stable habitats (Chapter 1) (Townsend *et al.* 1997a, Usseglio-Polatera *et al.* 2000). Regarding biological traits, large body size, long life span and less-than-one reproductive cycle per year have commonly been taken as evidence of relatively stable habitats with a low flood disturbance duration, high salinity and organic nutrient concentration (Chapters 3 and 4) (Townsend and Hilldrew 1994, Mellado *et al.* 2008). Besides, high diversification of locomotion and feeding habits are favored by the stability at confined wetlands (Heino 2008) (Fig. 25B).

(C)Biodiversity patterns

The response of aquatic biodiversity to habitat conditions depends on the attribute analysed, as proved in Chapter 2 where the driving processes for abundance and richness of aquatic communities were discussed in detail. In general terms, the results from studies performed at different scales and attributes (Chapters 1, 2 and 3) were consistent with the Intermediate Disturbance Hypothesis, which predicts highest overall biodiversity at intermediate levels of a given disturbance factor (Connell 1978) (Fig. 25C).

At low hydrological connectivity levels (e.g., confined oxbow lakes) competitive exclusion by the dominant species arises (Connell 1978). Consequently, the taxonomic abundance and biodiversity of aquatic macroinvertebrates lowers (Chapters 1 and 2), as trait diversity does (Chapters 3 and 4). On the other hand, the combination of stable environmental conditions and low macroinvertebrate abundance allowed zooplankton communities to reach high density and richness (Chapter 2).

At high hydrological connectivity levels (e.g., the river channel, secondary channels and backwaters) macroinvertebrate species abundance and richness were the highest (Chapter 1 and 2), as trait richness was (Chapter 3). Accordingly to Scarsbrook and Townsend (1993), the combination of moderate disturbance events (limited by river regulation) and high nutrient availability (increased by agriculture land-use) in river-floodplain habitats provides high habitat heterogeneity and resources availability to invertebrates, thus promoting their richness. Regarding plankton assemblages, high hydrological connectivity and macroinvertebrate pressure prevented plankton communities from reaching high density and richness (Baranyi *et al.* 2002).

Finally, at intermediate levels of hydrological connectivity (e.g., frequently flooded wetlands) macroinvertebrate species diversity and evenness, as well as trait diversity, were the highest (Chapters 1, 2 and 3). These highest values could be explained because at intermediate levels of disturbance both generalist and specialist species could coexist (Connell 1978). Moreover, under intermediate hydrological conditions, the highest phytoplankton abundance and richness was detected, which is related to the combination of low water turbulence and turbidity, and high nutrient availability that would exert a positive bottom-up effect on phytoplankton (Van der Brink and Van der Velde 1994, Heiler *et al.* 1995). In addition, other studies already highlighted the negative top-down effect of zooplankton on phytoplankton (Jeppesen *et al.* 1997, Vakkilainen *et al.* 2004), which could be a plausible explanation for the low phytoplankton abundance and richness observed in sites with low hydrological connectivity, where zooplankton had increased.

The Ebro in the context of other river-floodplains

Mediterranean floodplains are characterized by highly variable discharge with predictable torrential floods and severe droughts, while discharge is less variable in temperate floodplains (Bonada *et al.* 2007). Regarding arid and tropical systems, monsoonal discharge, aridity and high evapotranspiration losses characterize the former, while unpredictable torrential rainfall is typical in the latter (see rainfall-temperature diagrams, Fig. 3). These different climatic features, as well as other hydrological (timing, frequency, magnitude and duration of flood pulses) and environmental (geology, geomorphology, vegetation, water quality) characteristics may result in different aquatic structure (Poff and Ward 1990). Thus, it seems probable that patterns in aquatic communities observed in the Middle Ebro floodplain may not be applicable to rivers in other climatic areas. Herein, to find out if climatic restrictions result in different biological patterns, we gathered data from existing studies carried on four markedly different climatic areas, which allowed the comparison of relative macroinvertebrate patterns across the lateral hydrological connectivity (Table 19).

Table 19 Rivers used for comparison with the Ebro River. Arrangement of wetlands in a gradient of lateral hydrological connectivity (from 1 to 5) is based on habitat descriptions provided by the authors.

Climatic area* (River)	**Habitat type**					**Reference**
	1	**2**	**3**	**4**	**5**	
Mediterranean (Ebro)	River channel	Secondary channel	Backwater	Connected wetland	Confined wetland	Present study
Temperate (Tagliamento)	River channel	Secondary channel	Backwater	Connected wetland	Confined wetland	Arscott *et al.* (2005)
Arid (Cooper)	River channel	Secondary channel	Semi-perm wetland	Temp. wetland	Ephemeral wetland	Sheldon *et al.* (2002)
Tropical (Parana)	River channel	Secondary channel	Connected wetland	Isolated wetland	Temporal wetland	Zilli *et al.* (2008)

* Sampling method differed between the Mediterranean and arid regions (sweep net), and the temperate and tropical regions (grab). However, as patterns are analyzed within each region and not between regions, such difference is not considered to affect the present comparison.

In the first place, taxonomic differences between climatic regions were expected due to large-scale ecological differences and natural selection (Townsend and Hilldrew 1994). Consistently, Bonada *et al.* (2007) documented significant differences in the species composition of aquatic communities between temperate and Mediterranean rivers, which were mainly related to the historical frequency of disturbances (floods and droughts) as well as other climatic, geologic and geomorphic differences. In contrast, along a lateral hydrological connectivity, dominating invertebrate groups were similar in the Ebro and the temperate Tagliamento (Arscott *et al.* 2005) as well as in other temperate rivers such as the Rhône (Castella *et al.* 1991) or the Danube (Tockner *et al.* 1999b). In these ecosystems, aquatic insects dominated the main river channel and adjacent wetlands, while crustaceans and molluscs became more abundant in disconnected floodplain habitats. Nevertheless, differences appeared when compared to arid and tropical rivers, since crustaceans and molluscs dominated the main channel and adjacent wetlands of the arid Cooper Creek (Sheldon *et al.* 2002), and insects were found to be most abundant in temporal floodplain wetlands of the tropical Parana River (Zilli *et al.* 2008).

Secondly, regarding trait patterns across the lateral hydrological connectivity, no differences between river types were expected, as the traits needed to face hydrological disturbance (e.g., small body size, egg protection, short life-span, multivoltinism) should be similar. Certainly, trends in trait composition in the temperate Rhône River were in agreement with trait patterns in the Mediterranean Ebro River, reporting an increase in body size, number of reproductive cycles per year and tolerance to desiccation at decreasing levels of lateral hydrological connectivity (Dolédec and Statzner 1994) (Chapter 3 and 4). Despite this common pattern, higher frequency of droughts and floods in Mediterranean regions may result in a relatively higher abundance of traits related to disturbance resistance and resilience (Bonada *et al.* 2007). No information about multiple functional traits was available for arid and tropical ecosystems, but the distribution of functional feeding groups from the river channel to disconnected floodplain wetlands was consistent among the four climatic areas. In general terms, collector-gatherers and shredders dominated the river and adjacent wetlands, where they benefited from the abundance of algae and organic detritus. Predators became more representative at decreasing levels of hydrological connectivity possibly because of the closer biotic interaction (Heino 2008).

Finally, macroinvertebrate richness in both temperate and Mediterranean floodplains decreased from the main river channel to disconnected wetlands located further in their floodplains (Fig. 26). It is suggested that higher habitat heterogeneity and different sources of water in secondary channels and backwaters are responsible of their high macroinvertebrate richness (Castella *et al.* 1991, Foeckler *et al.* 1994). In addition, limitation of high-magnitude floods in regulated floodplains may allow different species to colonize adjacent wetlands (Scarsbrook and Townsend 1993). Similar patterns in macroinvertebrate richness across the lateral hydrological connectivity suggest that regardless of differences in discharge variability between temperate and Mediterranean regions, hydrological connectivity exerts a similar effect on aquatic communities. Consistent species and trait composition along the lateral hydrological connectivity lends support to this idea. If this is true, then general patterns in aquatic community structure and biodiversity reported for the Mediterranean Ebro River may be applicable to a broader range of climatic conditions, such as those characteristic of the temperate climate.

On the other hand, tropical and arid floodplains (Parana and Cooper respectively) showed almost the opposite of the pattern observed in the Mediterranean and temperate floodplains, with macroinvertebrate richness progressively increasing from the river channel to floodplain wetlands. Such a difference in richness patterns may be related to their extreme hydrological and environmental conditions (Puckridge *et al.* 1998) as well as to the temporality of their floodplain wetlands, which may foster organism blooms after flood episodes. However, other explanations, such as less human-altered floodplains in the arid and tropical zones, may not be disregarded. Nevertheless, further studies including data from a higher number of systems are needed to confirm and better understand such patterns.

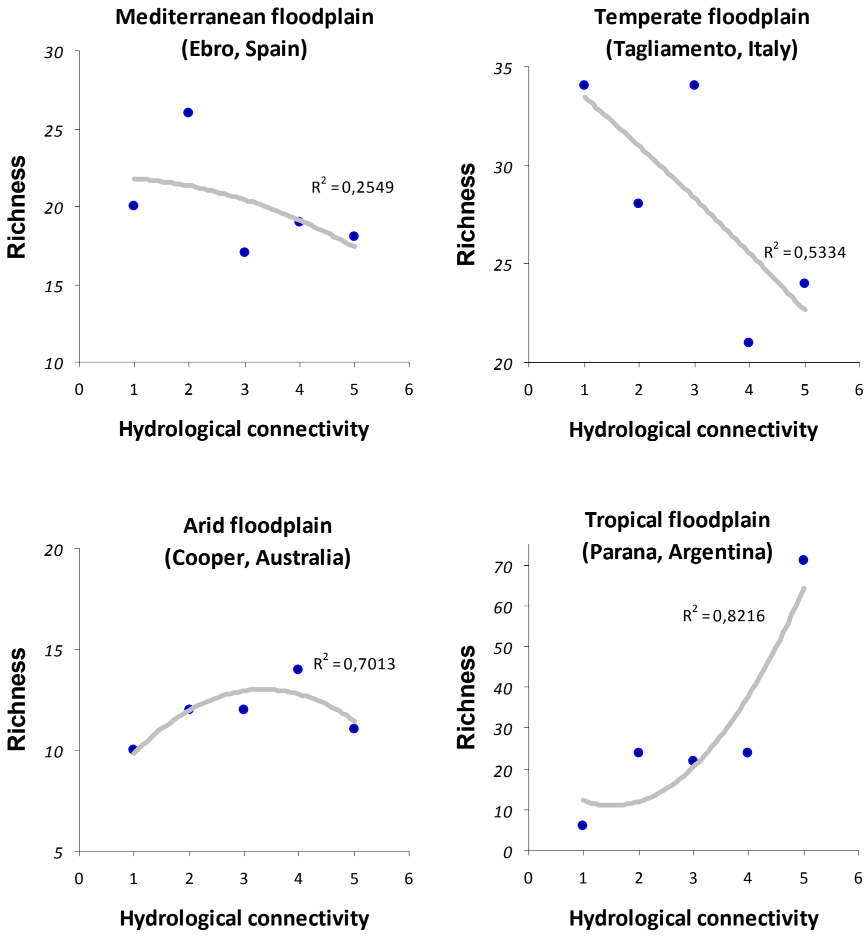

Figure 26 Patterns in macroinvertebrate species richness from the main river channel (1) to wetlands located further in the floodplain (5). See Table 20 for further description of habitats 1-5. Data have been extracted from Arscott *et al.* (2005), Sheldon *et al.* (2002) and Zilli *et al.* (2008).

Applications to the restoration of a regulated Mediterranean floodplain

Current trends in floodplain occupation and degradation suggest that ecological restoration of the floodplain habitat is essential for the preservation of the functions and benefits provided by the river ecosystem. Moreover, given the important role of hydrological connectivity, the future increase in water demand, combined with unpredicted impacts of climate change may have serious consequences on the aquatic communities of Europe and North-America rivers. For this reason, multidisciplinary studies, like the one presented here, may provide the necessary understanding of floodplain patterns and processes to increase the success of future management and restoration plans.

In the Middle Ebro River, the progressive regulation of river flow has altered the timing and reduced the frequency, duration and intensity of flood events that re-connect the floodplain habitats with the river channel. Consequently, a trend towards terrestrialization and landscape homogenization has been observed (Cabezas 2008), being one consequence the lack of wetlands showing intermediate degrees of hydrological connectivity (Chapter 2). Following Spänhoff and Arle (2007), restoration practices in degraded rivers, such as the Ebro, will lead to an increase in habitat heterogeneity, and therefore to an increased number of macroinvertebrate species and diversity. The ecological models developed in the present study support this observation and may help in establishing restoration targets for particular groups of organisms (Tockner *et al.* 2000). However, monitoring programs should be implemented in order to investigate further the efficiency of restoration activities, as well as to take new measures when ecological restoration fails.

So far, the diversification of the floodplain hydrological conditions, especially at the intermediate connectivity range (10-40 days yr^{-1}), seems to be the first objective of ecological restoration in the Middle Ebro River. Flood duration of existing wetlands can be modified through different restoration strategies (Buijse *et al.* 2002) (Table 20). The lowering, relocation or removal of embankments seem to be the easiest ways to restore the natural flood pulse at local scale, although the agreement of scientists, environmental managers and public opinion is still needed (Henry and Amoros 1995). For instance, in NOL2-4 a reduction in the flood limit (from 800-1200 to 400-600 $m^3\ s^{-1}$) through the removal of embankments and the re-opening of old connecting channels would lead to a tenfold increase of the actual flood duration (from 3-5 to 20-30 days yr^{-1}).

Table 20 Benefits and risks of restoration activities directed to increase the hydrological connectivity.

RESTORATION	BENEFITS	RISKS	POTENTIAL SITE OF APPLICATION
Manage river discharge according to climate variability Land-cover change	Diversify the morphological and hydrological conditions Help recuperate the natural riparian corridor	Increase risk in flooding human areas Lack of agreement between stakeholders and environmental managers	Ebro River catchment
Remove, relocate or lower embankments Re-open backwaters and connecting channels Digging new wetlands Lowering the floodplain height Dredge fine organic sediments	Increase habitat heterogeneity Increase species biodiversity Improve water quality Promote the recycling of organic matter Provide natural reservoirs of floodwater Reduce the potential negative effects of floods and droughts Provision of recreational areas	Increase sedimentation rates Dispersal of exotic species Eutrophication and pollution of floodplain habitats Impoverish of the river water quality if the flooded sector is not occupied by natural patches of vegetation Not self-sustaining system	Wetlands located within Natural Reserve Areas (NOL1-4 and COL1-2) Abandoned or unproductive areas of the floodplain (outside the Natural Reserves)

Another strategy to restore hydrological connectivity consists in the re-opening of old accreted river channels, and the creation of new wetlands in abandoned areas of the floodplain. As explained in Chapter 1, the digging of wetlands has proved to be a valid strategy in the Ebro, obtaining several benefits, such as the provision of new habitats for species (groundwater fed pools) different from the existing ones. Other riparian areas may be susceptible to use this restoration strategy, but newly-created wetlands should be self-maintaining and reproduce a natural diverse, functional system, a target which is not easy to accomplish, even after careful ecological design (Henry and Amoros 1995). In fact, re-opened habitats can soon close again due to high sedimentation rates and gravel deposition after spates.

Finally, it is worth highlighting that without proper management, an absolute increase in the hydrological connectivity would result in floodplain eutrophication and pollution (Heiler *et al.* 1995). Certainly, intense agricultural land-use in the Ebro catchment increased nitrate concentration in frequently flooded wetlands, impacting the taxonomic and functional composition of aquatic macroinvertebrates as well as their diversity (Chapters 1, 3 and 4). Water quality is a major component in controlling the aquatic ecosystem, and consequently, influences the success of restoration schemes. Higher retention times in floodplain wetlands after restoration may reduce nutrient load but such reduction may not be enough (Galat *et al.* 1998), hence other actions should be implemented. At local scale, natural diverse patches of terrestrial and aquatic vegetation provide a natural biological filter for river water flowing through the floodplain. At a higher scale, the control of the urban and industrial effluents combined with the progressive restoration of unproductive agricultural areas towards more natural vegetation patches would increase both landscape heterogeneity and water quality (Verhoeven *et al.* 2006). However, these actions should take into account not only the ecological but also the socio-economic context of the Ebro catchment (Cabezas 2008). For this reason, higher-scale management plans are needed to increase hydrological connectivity and reduce the nutrient and pollutant load of the Ebro, which would require the interdisciplinary cooperation between scientists and environmental managers to define the appropriate goals and procedures of restoration plans (Henry *et al.* 2002).

Conclusions

Photograph: Álvaro Cabezas

Conclusions

Main drivers of floodplain ecosystems

1. Lateral hydrological connectivity is the key factor structuring habitats and aquatic communities in the Ebro River ecosystem, which integrates several processes ranging from habitat re-organization to nutrient transport.
    - i. Wetlands in the Middle Ebro floodplain were arranged in a lateral hydrological connectivity, from wetlands closely related to the river dynamics and thus frequently flooded (e.g., secondary channels, backwaters) to those gradually isolated from the river dynamics (e.g., confined oxbow lakes).
    - ii. Different surrogates can be used to assess lateral hydrological connectivity. Flood duration appeared to be the best metrics to assess the macroinvertebrate community, whereas organisms with shorter life-cycles, such as zooplankton and phytoplankton, better responded to water level fluctuations.
2. Turbidity, salinity and nutrient status were secondary drivers, however important for understanding the response of aquatic communities. Distinctive water chemistry was reported in wetlands showing different hydrological connectivity:
    - i. Flooded wetlands (e.g., those just aside the main river and secondary channels) showed high turbidity and concentration of inorganic nutrients mainly coming from the river channel.
    - ii. Salts and organic nutrients accumulated in confined wetlands (e.g., oxbow lakes) during confinement periods between floods.
    - iii. Water chemistry of intermediately connected wetlands depended on the flood connexions frequency and duration.
3. Hydrological connectivity and environmental variability create a template for the aquatic community that enables predictions about the taxonomic and functional patterns in those aquatic communities more likely to occur under particular conditions.

Taxonomic patterns across the lateral hydrological connectivity

4. According to the Ebro template, wetlands arranged in a lateral hydrological connectivity showed the following differences in macroinvertebrate species composition:
    - i. Flooded wetlands were dominated by aquatic insect larvae and aquatic worms.

    ii. Non-insect taxa, such as crustaceans and gastropods dominated confined wetlands.

    iii. Constructed wetlands flooded through seepage were dominated by insects.

5. Consistently with the Intermediate Disturbance Hypothesis, macroinvertebrate species diversity peaked at intermediate levels of hydrological disturbance, where both generalist and specialist species can coexist.

6. The abundance and richness of three different groups of organisms (zooplankton, phytoplankton and macroinvertebrates) peaked at increasing degrees of hydrological connectivity (5, 30 and 40 days yr^{-1} respectively). This fact has been related to their different disturbance/stability preferences, their response to the specific physicochemical conditions presented by each habitat, and the potential biotic interactions among groups of organisms:

    i. Macroinvertebrate species abundance and richness were highest in the river channel, thus reflecting the high habitat heterogeneity and resource availability of this habitat. In addition, water discharge regulation in the Ebro River seemed to enhance the colonization of the river and adjacent wetlands.

    ii. Under intermediate hydrological conditions, the combination of low turbidity and turbulence, and high nutrient availability favored phytoplankton abundance and richness.

    iii. Zooplankton abundance and richness peaked in the most stable and confined sites, where zooplankton is protected from the disturbance effect of floods and also from macroinvertebrate predators, which are less abundant in confined wetlands.

Functional patterns across the lateral hydrological connectivity

7. Consistent with the taxonomic approach, hydrological connectivity was found to account for the highest amount of variability in macroinvertebrate trait composition, followed by physicochemical characteristics, which accounted for a low percentage of the trait variability, but were essential to understand the species adaptation to the floodplain hydrological and environmental conditions.

8. According to the Ebro template, wetlands arranged in a lateral hydrological connectivity showed distinctive macroinvertebrate trait composition, consistent with the River Habitat Templet:

    i. In frequently flooded wetlands, a diverse array of traits related to resistance (e.g., small body-shape, protection of eggs) and recovery from flood (e.g., short life-span, asexual reproduction) coexisted.

   ii. In contrast, confinement conditions in wetlands with less hydrological connectivity, resulted in a high representation of species showing the ability to interact with other organisms (e.g., large size, sexual reproduction), as well as to use effectively habitats and resources (e.g., diverse locomotion and feeding strategies).

9. Trait diversity was slightly higher in flooded than in confined sites, whereas trait richness was not significantly different. This suggests that the number of life-history strategies needed to persist in the face of disturbance remains more or less constant, only their relative dominance differs.

Application to the restoration of floodplain ecosystems

10. In the Middle Ebro River, progressive regulation of river flow has altered the natural river-floodplain interaction. Consequently, there are very few possibilities of new wetlands creation, while the diversity and functionality of the remaining ones are threatened by the limited exchange of water, nutrients and organisms between floodplain wetlands and the main river channel. Within this framework, our results underscore the importance of increasing the habitat diversity in degraded floodplains as a means to facilitate the recovery of the ecosystem biodiversity and functionality, so as to preserve the functions and benefits provided by the river.

11. The objective of degraded floodplains restoration in the Middle Ebro River should consist in re-establishing a wide range of wetland types in accordance with the river-floodplain potential for ecological sustainability. In order to achieve this, a number of actions can be positively performed, from the modification of embankments to the re-connexion of isolated floodplain habitats.

Conclusiones (Castellano)

Factores principales del ecosistema río-llanura

1. La conectividad hídrica lateral es el factor principal que estructura los hábitats y comunidades acuáticas del ecosistema río-llanura del Ebro, integrando varios procesos que van desde la reorganización de hábitats al transporte de nutrientes.
    i. Los humedales del Ebro Medio pueden ordenarse en un gradiente de conectividad hídrica lateral, desde humedales estrechamente relacionados con la dinámica fluvial y por tanto frecuentemente inundados (ej., brazos secundarios) a aquellos cada vez más aislados (ej., *galachos* confinados).
    ii. Entre los posibles indicadores existentes para medir la conectividad hidrológica, la duración de la riada es el más indicado para analizar la respuesta de macroinvertebrados, mientras que organismos con ciclos vitales más cortos, como zooplancton y fitoplancton, responden mejor a fluctuaciones en el nivel del agua.
2. La turbidez, salinidad y estado trófico son factores secundarios en el ecosistema río-llanura pero imprescindibles para comprender la adaptación de las comunidades acuáticas a la heterogeneidad natural de la llanura. Los humedales presentes en la llanura del Ebro Medio muestran características diferentes:
    i. Los humedales inundados con frecuencia muestran una elevada turbidez y concentración de nutrientes inorgánicos procedentes del río.
    ii. Sales y nutrientes orgánicos se acumulan en humedales confinados, durante los largos periodos entre inundaciones.
    iii. Las características físico-químicas de los humedales en un estado intermedio dependen de la duración y frecuencia de su inundación.
3. La combinación de conectividad hídrica y características físico-químicas conforman un marco espacio-temporal para el desarrollo de las comunidades acuáticas. Este marco permite predecir las características taxonómicas y funcionales con más probabilidades de ocurrir en determinadas condiciones ambientales (ej., en zonas de frecuente o por el contrario rara inundación).

Patrones taxonómicos a lo largo de un gradiente de conectividad hídrica lateral

4. De acuerdo con el marco desarrollado para el Ebro Medio, los humedales presentes en su llanura muestran las siguientes características taxonómicas:

    i. Los humedales inundados con frecuencia están dominados por larvas de insectos y oligoquetos.
    ii. En los humedales inundados raramente dominan los crustáceos y gasterópodos.
    iii. Los insectos son el taxón dominante en las comunidades presentes en humedales construidos, que se inundan a través del nivel freático.

5. De acuerdo con la Hipótesis de Perturbación Intermedia, la diversidad de macroinvertebrados en el Ebro Medio es máxima a niveles intermedios de perturbación (inundación), lo que permite la convivencia de especies generalistas y especialistas.

6. La abundancia y riqueza de tres grupos biológicos diferentes (zooplancton, fitoplancton y macroinvertebrados) es máxima a niveles crecientes de conectividad hídrica.
    i. La abundancia y riqueza de macroinvertebrados es máxima en el cauce fluvial, lo que refleja su gran heterogeneidad de hábitats y disponibilidad de nutrientes, así como la mayor posibilidad de colonización de este hábitat que permite la regulación de caudales.
    ii. En condiciones hídricas intermedias, la combinación de baja turbidez y turbulencia, y gran disponibilidad de nutrientes favorece el desarrollo del fitoplancton.
    iii. La abundancia y riqueza de zooplancton es máxima en los humedales más estables hidrológicamente y por tanto con menor conectividad hídrica. En estos ambientes el zooplancton se encuentra protegidos del efecto de inundaciones así como de la depredación por parte de macroinvertebrados, que son menos abundantes en este tipo de hábitats.

Patrones funcionales a lo largo de un gradiente de conectividad hídrica lateral

7. En concordancia con el enfoque taxonómico, la conectividad hídrica es el principal factor que explica la composición de rasgos biológicos en las comunidades acuáticas del Ebro Medio, si bien otros factores ambientales también juegan un papel importante.

8. Según el marco ecológico desarrollado para el Ebro Medio, los humedales presentes en su llanura de inundación presentan distintos rasgos biológicos:
    i. Los humedales inundados con frecuencia muestran una gran diversidad de rasgos biológicos relacionados con la resistencia a la inundación (ej., tamaño pequeño, protección de los huevos) y la rápida recuperación tras la inundación (ej., ciclos de vida cortos, reproducción asexual).

    ii. Por el contrario, es condiciones de confinamiento o baja frecuencia de inundación, otros rasgos relacionados con la interacción biótica (ej., gran tamaño, reproducción sexual) y el uso eficiente de los recursos disponibles (ej., varias técnicas de locomoción y alimentación) se vuelven más abundantes.

9. La diversidad de rasgos biológicos es ligeramente superior en humedales frecuentemente inundados que en aquellos más aislados, mientras que la riqueza de rasgos no es significativamente diferente. Esto sugiere que el número de estrategias biológicas necesarias para enfrentar la perturbación es contante, tan solo cambia su dominancia.

Aplicación a la restauración de ecosistemas río-llanura

10. En el Ebro Medio, la progresiva regulación de caudales ha alterado la relación natural entre el río y su llanura de inundación. Como consecuencia, la posibilidad de que se creen nuevos humedales de forma natural es cada vez menor, mientras que los que quedan pierden biodiversidad y funcionalidad debido al limitado intercambio de agua, nutrientes y organismos entre los diferentes elementos del ecosistema.
11. El objetivo de futuros planes de restauración en el Ebro Medio pasa por el re- establecimiento de un amplio rango de humedales de acuerdo con el potencial de la llanura para su desarrollo sostenible. Con este objetivo, diversas actuaciones pueden ser llevadas a cabo, desde la modificación de motas, o la creación de humedales artificiales, a la re-conexión de humedales aislados y re-naturalización de zonas abandonadas o poco productivas.

Conclusions (Català)

Factors principals per l'ecosistema riu-plana

1. La connectivitat hídrica lateral és el factor principal que estructura els hàbitats i comunitats aquàtiques de l'ecosistema riu-plana de l'Ebre, integrant diversos processos que van des de la reorganització d'hàbitats al transport de nutrients.
    i. Les zones humides de l'Ebre mitjà poden ordenar-se en un gradient de connectivitat hídrica lateral, des de zones humides estretament relacionades amb la dinàmica fluvial i per tant d'inundació freqüent (p.e. braços secundaris) a aquelles zones humides cada vegada més aïllades (p.e. *galachos* confinats).
    ii. Entre els possibles indicadors existents per mesurar la connectivitat hídrica, la durada de la riuada és el més indicat per analitzar la resposta dels macroinvertebrats, mentre que organismes amb cicles vitals més curts, com el zooplàncton i fitoplàncton, responen millor a canvis en el nivell de l'aigua.
2. La terbolesa, salinitat i estat tròfic son factors secundaris en l'ecosistema riu-plana, però són imprescindibles per comprendre l'adaptació de les comunitats aquàtiques a la heterogeneïtat natural de la plana d'inundació. Les zones humides presents en la plana d'inundació de l'Ebre mitjà mostren diferents característiques:
    i. Les zones humides amb una freqüència d'inundació elevada presenten una elevada terbolesa i concentració de nutrients inorgànics provinents del riu.
    ii. Les sals i els nutrients orgànics s'acumulen en les zones humides confinades, durant els llargs períodes entre inundacions.
    iii. Les característiques físiques i químiques de les zones humides en una situació intermèdia depenen de la duració i freqüència de la seva inundació.
3. La combinació de connectivitat hídrica i característiques físiques i químiques conformen un marc espaciotemporal per al desenvolupament de les comunitats aquàtiques. Aquest marc permet predir les característiques taxonòmiques i funcionals amb més probabilitat d'ocórrer en determinades condicions ambientals (p.e. en zones humides d'inundació freqüent, o pel contrari, en zones humides amb inundacions poc freqüents).

Patrons taxonòmics en un gradient de connectivitat hídrica lateral

4. D’acord amb el marc espaciotemporal desenvolupat per l’Ebre mitjà, les comunitats de macroinvertebrats de les zones humides presents a la seva plana d’inundació mostren les següents característiques taxonòmiques:
    - i. Les comunitats de les zones humides d’inundació freqüent estan dominades per larves d’insectes i oligoquets.
    - ii. Les comunitats de zones humides d’inundació poc freqüent es veuen dominades per crustacis i gasteròpodes.
    - iii. Els insectes són el taxó dominant en les comunitats presents en les zones humides construïdes, que s’inunden a traves del freàtic.
5. D’acord amb la Hipòtesi de Pertorbació Intermèdia, la diversitat de macroinvertebrats en l’Ebre mitjà és màxima a nivells intermedis de pertorbació (inundació), ja que aquestes condicions permeten la convivència de espècies generalistes i especialistes.
6. L’abundància i riquesa de tres grups biològics diferents (zooplàncton, fitoplàncton i macroinvertebrats) és màxima a diferent nivell de connectivitat hídrica.
    - i. L’abundància i riquesa de macroinvertebrats és màxima en el curs principal del riu, fet que reflexa la gran heterogeneïtat d’hàbitats i disponibilitat de nutrients, així com una major possibilitat de colonització d’aquest ambient a causa de la seva elevada connectivitat.
    - ii. En condicions hídriques intermèdies, la combinació de baixa terbolesa i turbulència, i gran disponibilitat de nutrients afavoreix el desenvolupament del fitoplàncton.
    - iii. L’abundància i riquesa de zooplàncton és màxima a les zones humides més hidrològicament estables presents en ambients més confinats i que tenen menor connectivitat. En aquests ambients els organismes zooplanctònics es troben protegits de l’efecte de les inundacions i de la predació per part dels macroinvertebrats, ja que són menys abundants en aquests tipus d’ambients.

Patrons funcionals en un gradient de connectivitat hídrica lateral

7. En concordança amb l’aproximació taxonòmica, la connectivitat hídrica és el principal factor que explica la composició dels trets biològics en les comunitats aquàtiques de l’Ebre mitjà, si bé altres factors ambientals també juguen un paper important.
8. Segons el marc espaciotemporal desenvolupat per l’Ebre mitjà, les zones humides de la seva plana d’inundació presenten uns trets biològics característics:

   i. Les zones humides d'inundació freqüent mostren una gran diversitat de trets biològics relacionats amb la resistència a la inundació (p.e. mida petita, protecció dels ous) i a una ràpida recuperació després de la inundació (p.e. cicle de vida curt, reproducció asexual).
   ii. Per contra, en condicions de confinament o de baixa freqüència d'inundació, altres trets relacionats amb la interacció biòtica (p.e. mida gran, reproducció sexual) i l'ús eficient dels recursos disponibles (p.e. diverses tècniques de locomoció i alimentació) es tornen més abundants.

9. La diversitat de trets biològics és lleugerament superior en zones humides d'inundació freqüent que en aquelles zones més aïllades, mentre que la riquesa de trets no és significativament diferent. Això suggereix que el nombre d'estratègies biològiques necessàries per afrontar la pertorbació és constant, i que tan sols canvia la seva dominància.

Aplicació a la restauració de l'ecosistema riu-plana

10. En l'Ebre mitjà, la progressiva regulació de cabal ha alterat la relació natural entre el riu i la seva plana d'inundació. Com a conseqüència, la possibilitat de que es creïn noves zones humides de manera natural és cada vegada menor, mentre que les que queden perden biodiversitat i funcionalitat a causa del limitat intercanvi d'aigua, nutrients i organismes entre els diferents elements de l'ecosistema.
11. L'objectiu de futurs plans de restauració en l'Ebre mitjà passa pel restabliment d'un ampli rang de zones humides d'acord amb el potencial de la plana d'inundació per al seu desenvolupament sostenible. Amb aquest objectiu, diverses actuacions es poden dur a terme, des de la modificació de motes, o la creació de zones humides, a la reconnexió de zones humides aïllades i a la restauració de zones abandonades o poc productives.

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Appendices

Photograph: Álvaro Cabezas

Appendix 1 Environmental characteristics of the Ebro river-floodplain. A: Environmental variables of sites monitored at the stretch-scale, B: Sites monitored at the Middle Ebro sector-scale.

A. Stretch scale

		RS (n=6)	**NOL1 (n=17)**	**NOL2 (n=15)**	**NOL3 (n=18)**	**NOL4 (n=5)**	**COL1 (n=11)**	**COL2 (n=4)**
Physic and Chemical variables								
Total Susp. Solids	TSS (mg L^{-1})	87.6 (81.1)	39.4 (33.8)	34.8 (33.4)	25.5 (15.7)	92.2 (123.3)	21.7 (14.1)	16.7 (13.6)
Total Diss. Solids	TDS (mg L^{-1})	1002.0 (304.3)	1779.3 (952.3)	1824.5 (459.2)	2021.3 (752.1)	1596.0 (766.2)	4375.3 (365.2)	1416.0 (135.7)
Alkalinity	HCO_3^- (mg L^{-1})	164.5 (87.0)	126.7 (68.9)	273.4 (71.4)	126.7 (25.7)	146.1 (46.5)	122.3 (54.5)	192.4 (18.2)
pH	Ph	8.1 (0.3)	8.0 (0.3)	7.7 (0.2)	7.8 (0.3)	7.9 (1.0)	7.8 (0.2)	8.1 (0.1)
Temperature	T (ºC)	14.5 (3.5)	16.4 (7.3)	13.1 (3.3)	15.9 (7.5)	15.3 (2.3)	17.2 (6.1)	16.2 (4.6)
Dissolved Oxygen	DO (mg L^{-1})	8.5 (2.7)	7.2 (2.0)	8.6 (1.7)	8.1 (3.6)	10.3 (9.3)	9.3 (1.5)	9.5 (2.0)
Bromine	Br^- (mg L^{-1})	0.2 (0.08)	0.2 (0.1)	0.5 (0.09)	0.2 (0.09)	0.09 (0.04)	0.6 (0.3)	0.07 (0.05)
Fluor	Fl^- (mg L^{-1})	0.2 (0.03)	0.1 (0.0)	0.1 (0.04)	0.3 (0.1)	0.2 (0.04)	0.3 (0.1)	0.2 (0.0)
Chlorine	Cl^- (mg L^{-1})	330.8 (102.2)	575.5 (392.1)	582.6 (101.7)	356.1 (123.4)	294.9 (98.1)	887.0 (190.9)	482.5 (54.5)
Sulphate	$SO_4^=$ (mg L^{-1})	502.4 (322.0)	720.9 (385.2)	534.4 (91.4)	1196.5 (487.3)	848.1 (562.1)	2353.3 (223.3)	431.5 (61.1)
Sodium	Na^+ (mg L^{-1})	166.6 (49.0)	285.6 (163.9)	306.6 (40.6)	183.6 (67.7)	157.4 (25.6)	442.6 (81.8)	250.1 (61.1)
Potassium	K^+ (mg L^{-1})	6.2 (2.9)	7.9 (2.0)	4.0 (1.0)	9.6 (2.0)	11.4 (2.9)	8.8 (1.6)	3.2 (0.2)
Calcium	Ca^{++} (mg L^{-1})	152.6 (69.7)	204.1 (70.2)	180.9 (16.0)	292.9 (91.9)	261.8 (115.8)	470.9 (97.3)	124.8 (25.9)
Magnesium	Mg^{++} (mg L^{-1})	28.4 (7.3)	41.8 (20.9)	41.9 (4.6)	48.2 (18.9)	31.8 (10.4)	110.0 (23.1)	25.4 (5.4)
Trophic variables								
Ash-free disolved matter	AFDM (mg L^{-1})	13.9 (6.1)	8.1 (4.3)	14.2 (31.7)	15.6 (11.9)	20.3 (9.3)	6.1 (2.1)	5.4 (5.5)
Nitrate	NO_3^- (mg L^{-1})	16.0 (4.6)	1.8 (3.1)	42.9 (7.0)	1.2 (2.7)	2.1 (4.7)	62.3 (38.9)	5.0 (3.3)
Nitrite	NO_2^- (mg L^{-1})	0.2 (0.1)	0.02 (0.01)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	1.0 (0.1)	0.1 (0.1)
Phosphate	PO_4^+ (µ L^{-1})	5.0 (0.00)	5.0 (0.0)	5.0 (0.0)	5.0 (0.00)	91.8 (120.6)	10.9 (13.4)	5.0 (0.0)
Dissolved Organic Phosphorus	DOP (µ L^{-1})	19.2 (13.6)	5.2 (3.7)	4.1 (3.3)	13.6 (12.9)	76.3 (90.9)	3.4 (3.7)	0.0 (0.06)
Dissolved Organic Nitrogen	DON (mg L^{-1})	0.4 (0.5)	0.1 (0.2)	0.02 (0.08)	0.3 (0.2)	0.2 (0.5)	0.06 (0.1)	0.12 (0.1)
Chlorophyll a	Chl-a (µ L^{-1})	21.6 (18.3)	14.4 (17.2)	7.4 (11.2)	33.7 (26.3)	43.9 (29.9)	3.7 (3.3)	3.1 (2.1)

B. Middle Ebro sector scale

		RS1 (n=4)	RS2 (n=3)	RS3 (n=3)	SC1 (n=12)	SC2 (n=4)	SC3 (n=2)	BW1 (n=2)	BW2 (n=2)	BW3 (n=2)	BW4 (n=3)	BW5 (n=1)	NOL1 (n=9)	NOL3 (n=8)	NOL4 (n=3)	COL1 (n=5)	COL2 (n=5)	COL3 (n=7)
Physic and Chemical variables																		
Total Suspended Solids	TSS	105.3	124.0	169.6	104.2	29.5	55.2	83.2	84.75	50.4	35.5	127.0	43.4	31.4	216.6	73.1	74.5	20.9
	(mg L^{-1})	(44.1)	(5.9)	(118.5)	(43.9)	(0.6)	(6.2)	(25.0)	(32.2)	(40.7)	(13.6)		(35.5)	(12.8)	(163.6)	(133.5)	(132.5)	(7.0)
Total Dissolved Solids	TDS	1152.0	1154.7	1088.0	1265.0	1402.0	1048.0	1130.0	1122.0	1618.0	2329.3	800.0	2532.9	2373.5	898.7	3129.6	1729.6	1420.0
	(mg L^{-1})	(0.0)	(4.6)	(62.4)	(18.3)	(191.7)	(243.2)	(121.6)	(115.3)	(647.7)	(1143.1)		(984.1)	(100.6)	(60.0)	(1411.7)	(847.9)	(326.8)
Alkalinity	HCO$_3^-$	203.3	209.5	175.9	221.4	424.9	221.1	221.8	203.65	324.4	227.5	228.8	148.8	152.7	198.5	107.1	181.9	198.3
	(mg L^{-1})	(20.8)	(28.7)	(5.1)	(59.5)	(255.1)	(38.0)	(23.2)	(23.55)	(119.6)	(17.5)		(89.2)	(52.9)	(21.8)	(60.9)	(54.5)	(19.4)
pH	pH	8.3	8.3	7.8	8.1	8.3	7.9	8.0	8.0	7.3	7.6	8.0	8.2	7.9	7.5	7.9	7.8	8.0
		(0.1)	(0.0)	(0.5)	(0.03)	(0.2)	(0.2)	(0.0)	(0.0)	(0.04)	(0.4)		(0.6)	(0.2)	(0.17)	(0.4)	(0.4)	(0.1)
Temperature	T	19.1	19.7	19.5	19.8	24.5	19.7	20.1	20.05	17.7	20.0	24.6	22.6	20.7	17.7	21.1	20.6	21.5
	(ºC)	(1.5)	(2.4)	(1.7)	(1.5)	(6.5)	(6.9)	(3.7)	(3.61)	(4.0)	(1.4)		(1.7)	(3.4)	(0.46)	(2.9)	(2.7)	(2.9)
Dissolved Oxygen	DO	7.7	8.2	7.4	7.9	12.5	9.0	6.2	6.70	4.8	10.8	4.7	6.7	7.0	0.24	9.3	8.0	8.5
	(mg L^{-1})	(0.1)	(0.8)	(0.2)	(0.2)	(4.9)	(2.4)	(0.6)	(1.39)	(0.4)	(4.9)		(1.2)	(0.5)	(0.09)	(0.4)	(0.7)	(0.9)
Bromine	Br$^-$	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.30	0.2	0.3	0.2	0.2	0.3	0.07	0.2	0.1	0.1
	(mg L^{-1})	(0.001)	(0.006)	(0.003)	(0.04)	(0.0)	(0.04)	(0.04)	(0.04)	(0.003)	(0.1)		(0.1)	(0.1)	(0.01)	(0.1)	(0.0)	(0.04)
Fluor	Fl$^-$	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.17	0.2	0.1	0.1	0.2	0.2	0.15	0.2	0.2	0.2
	(mg L^{-1})	0.005)	(0.005)	(0.02)	(0.006)	(0.0)	(0.007)	(0.1)	(0.08)	(0.03)	(0.03)		(0.1)	(0.1)	(0.02)	(0.05)	(0.0)	(0.03)
Chlorine	Cl$^-$	400.0	398.6	537. 9	367.6	285.7	225.2	289.1	615.0	256.9	736.0	246.2	482.3	481.0	182.8	302.1	346.4	425.7
	(mg L^{-1})	(45.8)	(42.1)	(452.9)	(54.5)	(17.2)	(38.0)	(39.5)	(301.6)	(2.2)	(241.9)		(252.6)	(138.7)	(29.0)	(104.5)	(125.0)	(90.7)
Sulphate	SO$_4^=$	372.1	370.7	479.7	364.1	345.2	272.7	399.2	1026.6	350.1	1164.7	353.3	987.4	802.4	221.0	310.8	340.8	921.8
	(mg L^{-1})	(15.8)	(16.0)	(239.6)	(19.3)	(9.3)	(64.6)	(24.5)	(339.3)	(18.6)	(479.3)		(474.6)	(476.9)	(78.0)	(101.1)	(92.5)	(461.3)
Sodium	Na$^+$	221.6	218.4	275.2	227.2	192.6	129.8	207.2	335.7	151.6	345.7	121.9	229.1	216.1	129.1	155.9	180.8	250.5
	(mg L^{-1})	(6.4)	(0.02)	(161.9)	(10.5)	(14.1)	(41.3)	(93.3)	(111.2)	(39.7)	(81.3)		(95.3)	(91.5)	(3.45)	(25.6)	(48.7)	(55.3)

(cont.)

		RS1 (n=4)	RS2 (n=3)	RS3 (n=3)	SC1 (n=12)	SC2 (n=4)	SC3 (n=2)	BW1 (n=2)	BW2 (n=2)	BW3 (n=2)	BW4 (n=3)	BW5 (n=1)	NOL1 (n=9)	NOL3 (n=8)	NOL4 (n=3)	COL1 (n=5)	COL2 (n=5)	COL3 (n=7)
Potassium	K^+	5.1	4.4	4.6	5.6	6.2	2.6	6.9	8.13	3.2	8.1	2.8	8.0	5.1	8.54	5.5	7.6	5.8
	(mg L^{-1})	(0.1)	(1.5)	(1.7)	(1.6)	(3.5)	(0.7)	(3.0)	(1.13)	(0.7)	(1.2)		(1.5)	(3.3)	(1.10)	(1.7)	(2.9)	(1.6)
Calcium	Ca^{++}	121.9	121.1	181.8	129.6	132.2	109.2	155.8	297.5	139.6	241.5	111.8	237.6	191.0	136.0	124.0	128.5	332.6
	(mg L^{-1})	(10.8)	(8.1)	55.7)	(8.7)	(15.8)	(25.2)	(40.7)	(29.6)	(32.4)	(42.2)		(77.9)	(86.4)	(9.70)	(8.0)	(17.0)	(165.4)
Magnesium	Mg^{++}	31.4	30.3	32.5	31.0	28.6	18.6	35.7	71.9	21.0	65.5	16.9	39.2	38.0	20.3	24.9	27.5	45.3
	(mg L^{-1})	(1.7)	(3.5)	(4.9)	(2.5)	(1.7)	(4.4)	(16.0)	(16.3)	(4.8)	(30.8)		(15.6)	(13.1)	(0.77)	(5.8)	(5.0)	(16.0)
Trophic variables																		
Ash-free	AFDM	12.4	9.4	13.2	10.3	29.7	22.0	11.1	17.4	24.4	37.3	15.7	26.7	68.5	30.6	33.9	17.9	37.8
Dissolved Matter	(mg L^{-1})	(3.9)	(1.9)	(5.4)	(2.8)	(5.5)	(4.5)	(3.9)	(5.52)	(0.5)	(19.6)		(11.6)	(6.2)	(33.6)	(12.9)	(16.9)	(24.3)
Nitrate	NO_3^-	24.1	24.3	11.5	22.0	18.7	8.1	10.3	9.88	16.9	2.2	16.0	0.01	23.2	0.02	19.1	19.3	0.4
	(mg L^{-1})	(1.1)	(0.6)	(3.3)	(3.1)	(3.0)	(11.4)	(14.5)	(13.63)	(0.4)	(2.7)		(0.01)	(17.0)	(0.0)	(11.7)	(10.8)	(1.0)
Nitrite	NO_2^-	0.03	0.03	0.1	0.1	0.1	0.2	0.01	0.03	0.3	0.1	0.3	0.005	0.03	0.01	0.02	0.1	0.05
	(mg L^{-1})	(0.02)	(0.02)	(0.002)	(0.1)	(0.1)	(0.3)	(0.009)	(0.03)	(0.2)	(0.1)		(0.0)	(0.03)	(0.0)	(0.009)	(0.2)	(0.1)

Appendix 2 Taxa list of species present in monitored habitats of the Ebro river-floodplain. A: macroinvertebrates by habitat, B: zooplankton by habitat, C: phytoplankton by habitat.

A. Macroinvertebrates by habitat

Taxonomic Group	Species	RS	SC	BW	NOL	COL
Hirudinea	*Helobdella staganalis*	+	+			
	Erpobdella sp.	+	+			
Oligochaeta	(e.g. *Nais* sp.)	+	+	+	+	+
Gastropoda	*Ferrissia* sp.		+		+	+
	Physa sp.	+	+	+	+	+
	Corbicula sp.			+		
	Galba truncatula		+			
	Gyraulus sp.	+				
	Stagnicola sp.		+	+		
	Theodoxus fluvialistis	+	+			
	Unio sp.		+			
Crustacea	*Atyaephyra desmaresti*	+	+	+	+	
	Procambarus clarkii		+	+	+	+
	Echinogammarus sp.	+	+	+	+	
	Gammarus sp.	+				
	Proasellus meridianus	+	+		+	+
	Argulus sp.					+
Insecta	*Baetis fuscatus*	+	+	+	+	+
(Ephemeroptera)	*Caenis luctuosa*	+	+	+	+	+
	Cloeon inscriptum	+			+	+
	Ecdyonurus sp.	+	+	+		
	Electrogena sp.	+				
	Ephemera sp.	+	+			
	Ephoron virgo	+				
	Thraulus sp.	+				
	Leptophlebia sp.	+	+			
Insecta	*Dytiscus* sp.				+	
(Coleoptera)	*Gyrinus* sp.					+
	Noterus sp.				+	
	Helochares sp.			+	+	+
Insecta	*Coenagrion scitulum*	+	+	+	+	+
(Odonata)	*Boyeria irene*	+			+	
	Trithemis annulata				+	+
Insecta	*Micronecta* sp.	+	+	+	+	+
(Heteroptera)	*Cymatia* sp.					+
	Gerris sp.		+	+		
	Glaenocorixa sp.					+
	Hidrometra sp.					+
	Mesovelia vittigera				+	+
	Microvelia reticulata				+	+
	Parasigara sp.					+
	Velia sp.				+	
Insecta	*Ecnomus* sp.	+	+	+	+	+

Taxonomic Group	Species	RS	SC	BW	NOL	COL
(Trichoptera)	*Glossosoma* sp.	+	+	+	+	
	Cheumatopsyche lepida	+				
	Lepidostoma sp.				+	
	Polycentropus sp.	+				
	Hydropsyche sp.	+				
	Metalype fragilis				+	
	Agraylea sexmaculata				+	
Insecta	Ceratopogonidae		+	+	+	+
(Diptera)	Culicidae				+	
	Eriopterini				+	
	Ephydridae					+
	Hexatomini		+			
	Tipulidae		+	+	+	
	Limoniidae		+			
	Chironomidae	+	+	+	+	+

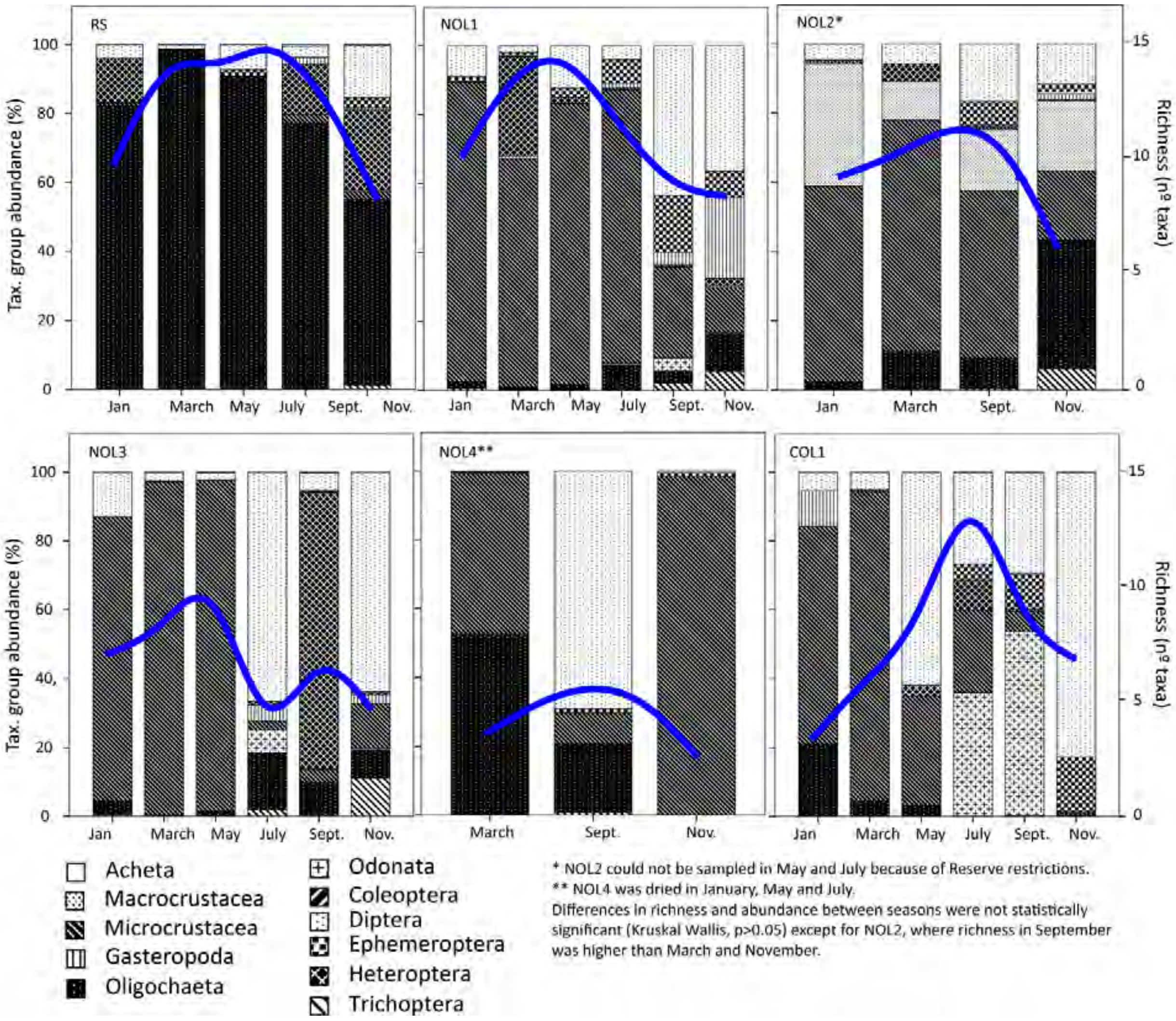
B. Zooplankton by habitat

Taxonomic Group	*Species*	RS	SC	BW	NOL	COL
Copepoda	*Acanthocyclops trajani*	+	+	+	+	+
	Macrocyclops albidus				+	
	Arctodiaptomus wierzejskii				+	+
Rotifera	*Notholca acuminata*				+	
	Polyarthra sp.				+	
	Keratella quadrata				+	+
Branquiopoda	*Daphnia sp.*		+	+	+	+
	Euricercus (E.) lamellatus	+			+	
	Ilyocriptus sordidus	+	+	+	+	+
	Simocephalus vetulus	+	+		+	+
	Chydorus sphaericus				+	
	Brachionus quadrangularis				+	
	Alona sp.				+	
	Bosmina sp.				+	
Ostracoda	*Bradleycypris oblicua*	+		+	+	+
	Darwinulidae				+	

C. Phytoplankton by habitat

Taxonomic Group	Specie	NOL	COL
Bacillariophyceae	Diatoms (e.g., *Gyrosigma* sp., *Nistchia* sp.)	+	+
	Rhizosolenia sp.	+	
Chlorophyta	*Scenesdesmus* sp.	+	
	Crucigenia sp.	+	
	Chloroccoccum sp.	+	+
	Gloeotila sp.	+	
Charophyta	*Filamentous green- algae*	+	+
Euglenoidea	*Euglena* sp.	+	
Chrysophyceae	*Chromulina* sp.	+	+
	Ochromonas sp.	+	
Cryptophyceae	*Cryptomonas* sp.	+	+
Dinophyceae	*Peridinium* sp.	+	+

Appendix 3 Temporal trends in macroinvertebrate species composition and richness over time in five floodplain wetlands and the Ebro River monitored in 2006.



* NOL2 could not be sampled in May and July because of Reserve restrictions.
** NOL4 was dried in January, May and July.
Differences in richness and abundance between seasons were not statistically significant (Kruskal Wallis, p>0.05) except for NOL2, where richness in September was higher than March and November.

Appendix 4 R statistical comands used through this study. A-D: functions used in Chapters 1 to 4.

R 2.5.1 is a free software (http://www.R-project.org/) for statistical computation and graphics.

A. Chapter 1

Data Input: Reads a file in Excell table format and creates a data frame from it, with cases corresponding to lines and variables to fields in the file.

```
data <- read.table ("clipboard", header=T, dec=",")      # Load the data
```

header = T The first line contains the column names

dec = ”,” Comas are used to separate decimals

```
 attach (data)   # Make variables accessible by name
 names (data)  # Get a list of the variables names
```

Normality Test

```
shapiro.test (var1)        # Perform Shapiro-Wilks test of normality
```

var1 = Variable whose normality is to be tested

$p > 0.05$ Means that the variable has a normal distribution

$p < 0.05$ Means that the variables has not a normal distribution

Variation partitioning

```
RDApartition <- varpart (species.hell, environmental.ln, hydrological.ln,scale=TRUE)
```

Test of fraccions:

```
anova.cca (rda (species.hell, environmental.ln, hydrological.ln))
anova.cca (rda (species.hell, hydrological.ln, environmental.ln))
anova.cca (rda (species.hell, environmental.ln))
```

Kruskal-Wallis rank sum Test

```
kruskal.test (var1 ~ factor, na.action = na.exclude)       # Check significant differences
```

var1 = response variable

factor = variable dividing cases into groups

$p < 0.05$ Means that significant differences are expected between factors

```
boxplot (var1 ~ factor, notch = T)          # Box and Wisklers Plot
```

notch = T # A notch is drawn in each side of the boxes. If the notches of two plots do not overlap this is strong evidence that the two medians differ

Generalized Additive Models (GAM)

library (mgcv) # Load library

GAM1 <- gam (var1 ~ s(var2,k=4) + s(var3,k=4) + …, family = quasipoisson) # GAM formula

var1= response variable

var2, var3= explanatory variables

k= maximum number of degrees of freedom

family= “quasipoisson”, “poisson”, “gaussian”, specifies the distribution and link to use in fitting the response variable.

gam.check (GAM1) # Diagnosis information about fitting procedure and results

anova (GAM1, GAM2, test = ”Chisq”) # Likelihood test

plot (GAM1, residuals = T, main=”…”) # Plot the GAM model

B. Chapter 2

Data Input: Reads a file in table format and creates a data frame from it, with cases corresponding to lines and variables to fields in the file.

data<-read.table ("clipboard", header=T, dec=",") # Load the data

header = T. The first line contains the column names

dec = ”,” . Comas are used to separate decimals

attach (data) # Make variables accessible by name

names (data) # Get a list of the variables names

Normality Test

shapiro.test (var1) # Perform Shapiro-Wilks test of normality

var1= Variable whose normality is to be tested

$p > 0.05$ Means that the variable has a normal distribution

$p < 0.05$ Means that the variables has not a normal distribution

Correlation Test

cor.test (var1, var2, method="spearman", use="pairwise.complete.obs")

var1, var2= variables to be correlated

method= “pearson”, “spearman” or “kendall”

use= “pairwise.complete.obs”

Generalized Additive Models (GAM)

```
library (mgcv)   # Load library
```

```
GAM1 <- gam (var1 ~ s (var2,k=4) + s(var3,k=4) + …, family = quasipoisson)      # GAM formula
```

var1= response variable

var2, var3= explanatory variables

k= maximum number of degrees of freedom

family= “quasipoisson”, “poisson”, “gaussian”, specifies the distribution and link to use in fitting the response variable.

```
gam.check (GAM1)       # Diagnosis information about fitting procedure and results
```

```
anova (GAM1, GAM2, test = ”Chisq”)   # Likelihood test
```

```
plot (GAM1, residuals = T, main=”…”)   # Plot the GAM model
```

C. Chapter 3

Data Input: Reads a file in table format and creates a data frame from it, with cases corresponding to lines and variables to fields in the file.

```
data<-read.table ("clipboard", header=T, dec=",")        # Load the data
```

header = T. The first line contains the column names

dec = ”,” . Comas are used to separate decimals

```
attach (data)   # Make variables accessible by name
names (data)   # Get a list of the variables names
```

Normality Test

```
shapiro.test (var1)        # Perform Shapiro-Wilks test of normality
```

var1= Variable whose normality is to be tested

p > 0.05 Means that the variable has a normal distribution

p < 0.05 Means that the variables has not a normal distribution

Correlation Test

```
cor.test (var1, var2,method="spearman",use="pairwise.complete.obs")
```

var1, var2= variables to be correlated

method= “pearson”, “spearman” or “kendall”

use= “pairwise.complete.obs”

Kruskal-Wallis rank sum Test

```
kruskal.test (var1 ~ factor, na.action = na.exclude)       # Check significant differences
```

var1 = response variable

factor = variable dividing cases into groups

$p < 0.05$ Means that significant differences are expected between factors

```
boxplot (var1 ~ factor, notch = T)         # Box and Wisklers Plot
```

Forward selection of variables for multivariate analyses

```
library (packfor)          # Load library
model1<- forward.sel (TaxaAbun, Environ, alpha=0.1)
```

Forward selection of variables by permutation of residuals under reduced model.

Environ = Data matrix containing *m* environmental variables for the *n* sites.

TaxaAbun = Data matrix containing the abundance of *p* species at *n* sites.

Alpha = Significance level for the inclusion of variables

Redundancy Analysis (RDA)

```
Library (vegan) # Load library.
Taxa.ln <- log1p (TaxaAbun)                # log (X+1) transformation of species data
```

TaxaAbun= Data frame containing the abundance of each taxa in each sampling site

```
Environ.ln<-log1p (Environ)                # log (X+1) transformation of environmental data
```

Environ= Data frame containing environmental variables

```
rda1 <- rda (Taxa.ln, Environ.ln)          # RDA model formula
summary (rda1)          # Extract the results of the model (variance explained, eigenvalues, species
                          scores, site scores etc)
```

```
anova.cca (rda1, step=100, perm.max=1000)    # Permutation Test of the whole analysis
anova.cca (rda1, by = "axis", step=100, perm.max=1000)         # Permutation Test by axis
```

```
plot.cca (rda1, display = c("sp","lc","bp","cn"), main = "RDA on …")  # Plot RDA
sco.distri (var1, factor, y.rank = T, labels = names (factor), grid = FALSE, sub="…")
```

Represents the mean and SD of a set of factors on a numeric score

D. Chapter 4

Data Input: Reads a file in table format and creates a data frame from it, with cases corresponding to lines and variables to fields in the file.

```
data<-read.table ("clipboard", header=T, dec=",")        # Load the data
```

header = T. The first line contains the column names

dec = ”,” . Comas are used to separate decimals

```
attach (data)    # Make variables accessible by name
names (data)    # Get a list of the variables names
```

Multivariate Analisis of Variance (MANOVA)

```
Man1 <- manova (Environ ~ factor)
```

Environ = Data matrix containing m environmental variables for the n sites.

factor = variable dividing cases into groups.

```
summary.manova (Man1, test = “Pillai”)
```

test = “Pillai”, “Wilks”, “Hotelling-Lawley” or “Roy”, test statistic to be used.

One-way analysis of variance (ANOVA)

```
summary.aov (var1 ~ factor)     # Perform analysis of variance
```

var1 = response variable.

factor = variable dividing cases into groups.

$p < 0.05$ Means that significant differences are expected between groups.

Fourth-corner statistics

```
library (fourthcorner) # Load library
fourth2<-fourthcorner (Environ, TaxaAbun, TaxaTrait, modeltype = 2, nrepet=999)
fourth4<-fourthcorner (Environ, TaxaAbun, TaxaTrait, modeltype = 4, nrepet=999)
```

Fourth-corner model formula

Environ = Data matrix containing m environmental variables for the n sites.

TaxaAbun = Data matrix containing the abundance of p species at n sites.

TaxaTrit = Data matrix containing s species traits for the p species

modeltype = 1-5 different permutation models available

nrepet= the number of permutations

```
summary (fourth2)       # Extract the results of the fourthcorner using permutation model 2
summary (fourth4)       # Extract the results of the fourthcorner using permutation model 4
```

Rao's diversity

```
library (ade4) # Load library
TaxaDist<- dist.quant (TaxaTrait, method=3)    # Computes distance matrices
```

TaxaTrait= Data matrix containing the *s* species traits for the *p* species.

Method = 1-3 indicates Canonical, Joreskog or Mahalanobis respectively.

```
divc (TaxaAbun, TaxaDist)        # Calculate Rao's diversity coefficients within samples
```

TaxaAbun= Data matrix containing the abundance of *p* species at *n* sites.

TaxaDist = Distance matrix

Wilcoxon Rank Sum Test

```
wilcox.test (var1 ~ factor)        # Perform non-parametric analysis of variance
boxplot (var1 ~ factor)            # Box and Wisklers plot
```

var1 = response variable

factor = variable dividing cases into groups

$p < 0.05$ Means that significant differences are expected between factors

Appendix 5 Photograpsh of monitored sites in the Ebro River.

Photo 1. The Ebro River in its middle stretch, near the city of Zaragoza.

Photo 2. Agricultural areas dominating 60% the Ebro floodplain. Extracted from www.kalipedia.com.

Photo 3. Collecting macroinvertebrates at the river channel.

Photo 4. Agricultural and urban areas affected by the Ebro 2007 overbank flood. Extracted from blogsdeopinion.blogspot.com.

Photo 5. Agricultural and urban areas affected by the Ebro 2007 overbank flood. Extracted from blogsdeopinion.blogspot.com.

Photo 6. NOL1 during a flood episode (2,282 m^3s^{-1}, 10-yr return period) occurring in 2007.

Photo 7. NOL2 during a flood episode (2,282 m^3s^{-1}, 10-yr return period) occurring in 2007.

Photo 8. NOL3 during a flood episode (2,282 m^3s^{-1}, 10-yr return period) occurring in 2007.

Photo 9. COL creation within natural reserve areas and plantation of riparian species.

Photo 10. COL creation in abandoned areas of the floodplain to mitigate wetland loss.

Agradecimientos

Hace ya cinco años que llegué a Zaragoza desde Salamanca, una soriana, logroñesa de adopción, con vocación investigadora. Con tal bagaje cultural, tenía que contar con las herramientas suficientes como para enfrentar un doctorado, o como poco para preparar un buen menú del norte. No ha sido fácil, tampoco ha sido siempre gratificante, sino que me ha llevado mucho tiempo y esfuerzo, pero gracias a la ayuda y paciencia de mucha gente finalmente aquí estoy, sentada mirando al frente sobre los tejados de Lyon, pensando en la cantidad de personas a quienes debo agradecer todo lo que me han enseñado y aguantado. La compañía en las salidas de campo, los análisis de laboratorio, las interminables horas delante del microscopio, las correcciones de los artículos, o simplemente las charlas filosófico-festivas a la hora de la merienda. No quisiera por ello olvidarme de ninguna de esas personas que han contribuido directa o indirectamente a que ahora acabe mi tesis doctoral sin volverme loca en el intento.

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Terminar la tesis implica un sentimiento contradictorio, porque tienes muchas de acabar, de conseguir el objetivo por el que llevas muchos años trabajando, de ser por fin doctor, de sentir que todo esto tiene un sentido, que ha valido la pena. Pero a la vez, echas la vista atrás y te das cuenta de que estas realmente a gusto entre tus compañeros, que te has acostumbrado a una rutina y no quieres dejarlo atrás, porque lo que viene delante es desconocido, y solo por eso da miedo. Yo no sé que me depara el futuro, si lograré continuar con la carrera investigadora, o como tantos otros me quedaré en el camino. Solo espero encontrarme con mas Alvaros, Eduardos, Marias, Marios, Pilis y tantos otros en los que apoyarme y de los que aprender. Solo espero volver algún día y encontrármelos en el IPE, en la Universidad, en el bar o en la frutería, y que sean siendo los mismos. Animo chicos!!