Ecology of the benthic macroinvertebrates in the lower Ebro River: community characterization, population dynamics and bioaccumulation of pollutants in response to environmental factors

Ecologia dels macroinvertebrats bentònics al tram baix del riu Ebre: caracterització de la comunitat, dinàmica de poblacions i bioacumulació de contaminants en resposta a factors ambientals

Núria Cid Puey

Núria Cid Puey

Ecology of the benthic macroinvertebrates in the lower Ebro River: community characterization, population dynamics and bioaccumulation of pollutants in response to environmental factors

Ecologia dels macroinvertebrats bentònics al tram baix del riu Ebre: caracterització de la comunitat, dinàmica de poblacions i bioaccumulació de contaminants en resposta a factors ambientals

Departament d'Ecologia
Universitat de Barcelona

2010

IRTA
Ecosistemes Aquàtics

The effects of the main anthropogenic impacts in the fluvial ecosystem of the lower Ebro River are evaluated along the five chapters of the present thesis, using the benthic macroinvertebrate community as a bioindicator. Different approaches are integrated in function of the type of stressor, considering mainly heavy metal and organochlorine pollution and hydrological alterations. These approaches focus on the taxonomy and biological traits of the community, and on the bioaccumulation of pollutants, population and life history studies on keystone species such as *Ephoron virgo* (Ephemeroptera: Polymitarcyidae). The results evidence a strong ecological response, and demonstrate that these effects act at different levels of organization, including communities, populations and individuals.

Els efectes dels principals impactes antropogènics de l'ecosistema fluvial del tram baix del riu Ebre s'estudien al llarg dels cinc capítols d'aquesta tesi, utilitzant la comunitat de macroinvertebrats bentònics com a bioindicadors. Depenent del tipus d'estrès, tenint en compte principalment la contaminació per metalls pesants i organoclorats i les alteracions hidrològiques, s'han considerat diferents aproximacions. Concretament, l'estudi se centra en la taxonomia i els trets biològics de la comunitat, la bioacumulació de contaminants, i els estudis poblacionals i relacionats amb el cicle biològic d'espècies clau com l'*Ephoron virgo* (Ephemeroptera: Polymitarcyidae). Els resultats evidencien una resposta ecològica important, i demostren que aquests efectes actuen a diferents nivells d'organització, incloent comunitats, poblacions i individus.

ncid@ub.edu

TESI DOCTORAL

Departament d'Ecologia

UNIVERSITAT DE BARCELONA

Programa de doctorat: Ecologia Fonamental i Aplicada

Bienni 2004-2006

Ecology of the benthic macroinvertebrates in the lower Ebro River: community characterization, population dynamics and bioaccumulation of pollutants in response to environmental factors

Ecologia dels macroinvertebrats bentònics al tram baix del riu Ebre: caracterització de la comunitat, dinàmica de poblacions i bioacumulació de contaminants en resposta a factors ambientals

Memòria presentada per

Núria Cid Puey

Per optar al grau de

Doctora per la Universitat de Barcelona

Barcelona, juliol de 2010

Vist-i-plau dels directors de la tesi

Dr. Carles Ibáñez i Martí
Director de la Unitat
d'Ecosistemes Aquàtics
IRTA

Dr. Narcís Prat i Fornells
Catedràtic del Dept. d'Ecologia
Universitat de Barcelona

Als meus pares, per ser tan autèntics

"L'aigua lliscava amb indiferència entre el silenci de trinxeres buides, filferrades menjades pel rovell, cases desfetes, terra llaurada per les bombes. Tanta porfídia i tanta mort –pensava el Nelson– no havien alterat la seva passa: les pluges de tardor i de primavera l'havien inflat, les ardorades estivals l'enflaquien, però les aigües no guardaven la memòria de la batalla. La memòria es cosa dels hòmens; ell, l'Ebre, era una força insensible als afanys d'aquella gent que li capturava els peixos, l'esgallava amb les quilles de les naus o trobava la mort en les seves entranyes fangoses i fredes".

Jesús Moncada, *Camí de Sirga*

Agraïments

Aquest treball no hagués sigut possible sense l'ajuda i el recolzament de moltes persones, tan a nivell professional com personal.

Sobretot vull agrair als meus directors, Carles Ibáñez i Narcís Prat, el seu suport tant al principi, de la tesi com al llarg de l'elaboració i redacció d'aquest treball, i sobretot per donar-me l'oportunitat de poder haver viscut aquests anys tan intensos i enriquidors. Gràcies Narcís per guiar-me encara que fos des de la distància i per ser tan directe i clar, pel teu *swing*, i per treure el temps de davall les pedres i donar-me els ànims que necessitava en l'esprint final. Gràcies a Carles per donar-me llibertat i no dubtar del meu treball, per animar-me a viatjar i per fer que els becaris tinguéssim un espai de treball més digne.

Gràcies a mons pares, que sempre m'han recolzat en tot el que he fet. A ma mare, la persona més forta que he conegut, gràcies per donar-me força i fe en mi mateixa, i per saber escoltar. A mon pare, per ensenyar-me a respectar i estimar la natura i a no tindre-li por, a fer volar la imaginació i veure que hi ha camins alternatius. I a tota la meua família, al caliu dels iaos i iaes, que encara no saben ben bé què faig..., a l'estufa de llenya, a l'esgorfa i les tomates de penjar, als cocs de sagí i a les ametlles garrapinyades, als polos d'anís, i a l'olor de most. A casa. A la padrina extrovertida i la seua pintura, al padrí introvertit i la seua vinya, als tios i ties i cosins, i a la tia Joanita que té un cor immens, a la Laura, la Gemma i a la Martina que ens has sorprès amb la teva dolça i inesperada arribada. A Rosendo i la MªAngels, la Vero i l'Albert, que sou família. I als que ara ja són record.

Al llarg d'este camí, sempre he portat ben a dins els orígens gandesans i la Terra Alta. Em sento feliç d'haver crescut a un poble, de xalar pels carrers i jugar a la plaça i pels

sembrats, de no saber què és el fred ni la calor, ni les hores digitals. A les Olles i al Toll del Vidre del Canaletes i l'Algars, als grills, les caderneres, blauets i granotes, rodadits i sabaters, i als rius i l'aigua que ens donen la vida!

I al piano pacient, tu que m'has donat equilibri, alegria i disciplina, tant temps que no t'he fet el cas que et mereixies, ara ja ens toca una bona afinada als dos!

Vull agrair a tots els amics que m'han acompanyat al llarg d'estos anys per la seva paciència i bon humor, suport i comprensió. Als de tota la vida: Mª Carme, Laia i Antonio, 3 punts cardinals tant diferents i tan importants. Hi ha coses que no s'esborren mai i 30 anys junts fan que aquesta tesi també sigui una mica vostra. A l'Adrià i la Marta, els meus incondicionals de la facultat i grans amics. A Pau, tanta complicitat i tantes coses viscudes junts no hi caben entre estes línies, m'has fet riure tant! Gràcies a tots per ser-hi quan tos he necessitat, per animar-me, per recollir-me. A Rafel, per estar al meu costat durant els darrers 4 anys, per haver compartit bons moments i il·lusions, i acompanyar-me aquí i a l'altra punta de món, malgrat que ara haguem pres camins diferents. I a tota la colla d'amics de Gandesa: Agustí, Esther, Paco, Rut, Manel, Rafel, Jaume, Mando, Jose, Jaume, Chals, Raquel, Joaquin, Gemma, Jimi, Dolors i tothom, que som tants que no hi cabem!

A la gent del departament d'Ecologia, d'ara i d'abans, els que han marxat i els que encara hi sou, i sobretot als antics Ecobills que m'estimo tant (Pau, Blanca, Iraima, Mireia, Tura, Cesc, Miguel, Raúl, Núria B), a la càlida Silvia, a la Isabel, l'Eduard, l'Ainhoa, Dani, el Biel, Joan Gomà, Oriol, Gemma, Carles, Salva, Luciano, Jaime, Mary, Pere Renom i tots els que em deixo! em va saber molt greu marxar del vostre costat! I gràcies i Vitalik, per acompanyar-me a mostrejar i passar aquells anys tan bons a Barcelona. I a la meva companya de marató: Iraima, ja hem arribat a la fi! Quan diem que algo ho acabem, ho acabem...

Gràcies als amics que m'ha donat l'estada a l'IRTA, heu fet que el Delta i les Terres de l'Ebre siguin un gran lloc per viure. Rosa, companya i gran amiga,i tan diferents que som! David, gràcies per fer-me sentir rapitenca i acompanyar-me en les nostres aventures pel Guai quan vaig arribar i no coneixia a ningú, per saber xalar tant i riure-mon de natres mateixos. Maite, tanta connexió! has sigut i ets una persona lliure i esplèndida. Joaquin, viva la ironía y nuestras largas charlas y risas, te echamos de menos. Mireia, tanta energia i sentit de l'humor, ha sigut genial conèixe't. Gràcies a

David Mateu (Turro), que ha sigut l'ànima i suport de tots els mostrejos, ets un solet! A Andrea, ai nena quants anys i que ràpid han passat! i a Fon, que nos embarcaste en la taxonomía, valiente siendo el único becario entre tantas mujeres. I més gràcies, a Laia, Silvia, Rosa V, Carme R., Berta, Gloria, Rafa Sánchez, Miquel Àngel, Rosa T, Vanesa, Núria, Sandra, Anaïs, Cinta i Pili, i a tots els becaris, investigadors i tècnics, m'heu fet riure quan estava massa capficada i heu fet que el treball fos més enriquidor.

A la Maria Rieradevall, per ajudar-me en la identificació de quironòmids i donar-me ànims quan els arxius s'esborren sobtadament! A Rut Collado per identificar els oligoquets a espècie, a la Núria Bonada pels tricòpters i al Javier Alba-Tercedor per resoldre'm dubtes d'Efemeròpters. Gràcies al Sylvain Dolédec per donar un curs de trets biològics tan productiu a Coimbra.

I per no oblidar els que em van fer sentir com a casa durant l'estada Nova York, al David Strayer per ser tan entusiasta amb la seva feina i per acollir-me, a Heather Malcom per posar-me a punt, a Miquel Tuson per fer de *Cicerone* els caps de setmana i mostrar-me l'altra cara de la ciutat, i a Vanessa, Zara, Catherine, Jen, Michael, Lorena i la gent tan agradable del Cary Institute of Ecosystem Studies. A les persones que m'han acompanyat durant els últims mesos de la tesi a Coimbra, que han estat els més durs, gràcies Maria Joao, Manuel Graça, Josi, Joao, Filipe, Daniel, Heliana; Filipe M., i tothom de l'IMAR. Gràcies Pieter per cuidar de mi i ser el meu company, per ser tan espontani i lliure, i fer abraçades de gegant, espero que no canviïs mai.

En definiva, gràcies a tots i totes els que heu estat al meu costat mentre va durar tot això i per fer que aquest camí no hagi sigut tant difícil de recórrer!

La Ràpita, estiu del 2010

Aquest estudi ha estat finançat per la generalitat de Catalunya (Departament d'Innovació, Universitats i Empresa i Departament de Medi Ambient i Habitatge), el Fons Social Europeu I el Govern d'Espanya (Ministerio de Educación y Ciencia, projecte de recerca CGL2006-01487, Plan Nacional I+D+I; Ministerio de Medioambiente y Medio Rural y Marino; Consejo Superior de Investigaciones Científicas). Agraeixo també a la Confederació Hidrogràfica de l'Ebre la cessió de dades utilitzades al llarg d'aquesta tesi.

Contents

Resum (*in Catalan*)

INTRODUCCIÓ

Els ecosistemes són crucials per al benestar de les persones degut a la seva contribució a l'aprovisionament, al suport i regulació dels processos ambientals i als valors culturals. En aquest context, els rius proporcionen l'abastament d'aigua per a les activitats humanes bàsiques i de producció, com necessitats domèstiques, agricultura, indústria, producció d'energia, transport, pesca i altres activitats recreatives (Allan & Castillo, 2008). Com a resultat de la intensificació d'aquestes activitats humanes, el deteriorament dels ecosistemes d'aigua dolça ha estat particularment notable durant els darrers 50 anys, principalment a causa de l'alteració de processos naturals, de la sobreexplotació de recursos i de la contaminació. Els grans rius, especialment els seus cursos baixos, reben una àmplia varietat d'impactes humans on les principals pressions considerades són les alteracions hidrològiques i geomorfològiques, els usos del sòl, els contaminants i la introducció d'espècies no autòctones (Petts et al., 1993; Poff et al., 2007; Klok & Kraak, 2008; Strayer et al., 2008). És per aquest motiu que l'alteració o desaparició de l'hàbitat, canvis en estructura tròfica, i la disminució de la qualitat de l'aigua, han comportat canvis en processos de l'ecosistema i una debilitació biològica. A més, les prediccions de canvi climàtic (IPC, 2007) que pronostiquen una reducció de les precipitacions i un augment de la temperatura depenent de la regió en concret, així com les captacions d'aigua que hi ha projectades, augmentaran encara més la pressió sobre els rius. Per exemple, la Península Ibèrica ha estat inclosa dins d'una de les àrees més amenaçades en el futur pel que fa a la pressió sobre els recursos hídrics, la contaminació i l'erosió (Tockner et al., 2009).

La fauna dels hàbitats d'aigua dolça (que inclou invertebrats, amfibis, peixos i ocells) representa una riquesa de més de 82.500 espècies, de les quals el grup més divers correspon als invertebrats aquàtics (vegeu Tockner et al., 2009). Tanmateix, durant els darrers 40 anys, la fauna d'aigua dolça ha mostrat la disminució més dràstica de biodiversitat si es compara amb els ecosistemes terrestres i marins (Millenium Assessment, 2005). Amb l'augment de l'estrés, les espècies més tolerants i generalistes esdevenen dominants, mentre que les espècies més sensibles esdevenen més vulnerables o s'extingeixen. Com a resultat, es produeix una homogeneïtzació de la comunitat i una pèrdua de la diversitat i de les funcions de l'ecosistema. Aquest pèrdua funcional, reflectida en l'empobriment de les comunitats, pot tenir efectes negatius importants en els serveis que l'ecosistema ens proporciona, que a part d'influir en els rius, també afecta als ecosistemes estuarins i marins. Per tal de protegir els recursos dels ecosistemes aquàtics, la legislació ambiental europea va crear la Directiva Marc de l'Aigua de la UE (Comissió Europea, 2000), que considera la integritat ecològica de comunitats biològiques. Els objectius prioritaris d'aquesta directiva europea queden palesos a l'Article 1, segons el qual cada estat membre de la Unió Europea ha d'assolir un bon estat ecològic dels ecosistemes aquàtics abans de l'any 2015.

Els macroinvertebrats aquàtics s'han utilitzat freqüentment com a bioindicadors de diagnosi ambiental de l'aigua perquè exerceixen un paper clau en ecosistemes aquàtics i responen considerablement a les variables ambientals (Rosenberg & Resh, 1993). La seva gran biodiversitat i la seva contribució a l'aportació de biomassa a la cadena tròfica ha comportat que, des de principis del segle XX fins a avui dia, hagin estat uns organismes objecte d'estudi per a l'avaluació de la contaminació dels ecosistemes aquàtics (Bonada et al., 2006), i que s'hagin integrat en les polítiques mediambientals com un dels grups d'organismes més importants per definir-ne la integritat ecològica (Comissió Europea, 2000). A partir d'aquests organismes, s'han desenvolupat diverses línies metodològiques segons els objectius de l'estudi i el nivell d'organització. A nivell de comunitat, la composició taxonòmica dels macroinvertebrats ha estat l'eina principal per al seguiment de l'estat ecològic del rius, utilitzant índexs multimètrics basats en la tolerància a la contaminació de cada espècie (Bonada et al., 2004; Bonada et al., 2006) i protocols estandarditzats (ex., RIVPACS, GUADALMED). Més recentment, l'ús de diversos trets biològics dels invertebrats (ex. mida de cos, reproducció, dispersió) s'ha considerat com a eina alternativa o complementària, tenint en compte que els canvis en

la composició d'aquests trets tenen conseqüències en el funcionament de l'ecosistema (Dolédec et al., 1999; Statzner et al., 2005; Statzner & Beche, 2010). Aquest mètode que utilitza multiples trets biològics es basa en els conceptes del River Habitat Templet (Townsend & Hildrew, 1994) que prediu *a priori* la resposta de cada tret davant de pertorbacions naturals del medi, i estableix la base per predir impactes antropogènics en els cursos d'aigua (Bonada et al., 2006; Henle et al., 2006). Malgrat que els estudis que consideren com a nivell d'organització les poblacions o individus no siguin tan abundants com els basats a nivell de comunitat (Bonada et al., 2005), durant els darrers 10 anys han experimentat un augment significatiu, principalment pel que fa a estudis de la bioacumulació dels contaminants o d'ecotoxicologia (Hare et al., 2001; Barata et al., 2005; De Lange et al., 2005; Cain et al., 2006; Buchwalter et al., 2007) i a deformacions morfològiques en resposta als contaminants (Diggins & Steward, 1998; Bonada et al., 2005; Skinner & Bennet, 2007). Tanmateix, molts d'aquests mètodes que observen respostes a nivell d'organisme, no donen lloc a canvis en la comunitat. Per exemple, les deformitats del mentum en l'espècie tolerant *Chironomus riparius* Meigen, o els alts nivells de bioacumulació en *Hydropsyche* spp. no generen canvis en les seves abundàncies en llocs altament contaminats (Cain et al., 2004; De Haas et al., 2005). Malgrat que aquestes espècies de macroinvertebrats tolerants a la contaminació no contribueixin a canvis en la comunitat, aquest fet els dóna la capacitat d'actuar com a sentinelles de l'ecosistema i de detectar nivells i gradients de contaminació a escales espacials més grans. Llavors, com que les respostes als contaminants observats a nivell de comunitat poden ser consqüència dels efectes sobre espècies relativament sensibles (ex., *Ephoron virgo* [Olivier], vegeu Klook & Kraak, 2008), els mètodes a nivell individual i de població d'aquestes espècies en àrees impactades són crucials. Generalment, l'ús combinat d'una espècie tolerant i d'una sensible podria proporcionar una eina més integradora (Adams & Greeley, 2000), ja que la detecció de respostes a nivell de població d'espècies clau pot predir canvis en la comunitat o fins i tot a nivell de l'ecosistema.

Segons aquestes consideracions, aquesta tesi aborda diverses aproximacions (composició i mètrica de la comunitat, dinàmica i bioacumulació de contaminants en les poblacions) depenent de l'impacte humà considerat (ex. alteracions del cabal, contaminació, canvis de temperatura, presència d'espècies no autòctones) en el context del tram baix del riu Ebre.

Context de l'estudi: el tram baix del riu Ebre

El riu Ebre està situat al nord-est de la Península Ibèrica i té una conca de 85.362 km^2 amb una longitud de 910 km. És el riu més gran a Espanya en termes de descàrrega d'aigua (11,982 hm^3/ any al tram baix vegeu www.chebro.es), un dels rius més importants del Mediterrani, i una de les conques més grans d'Europa (Tockner et al., 2009). El riu Ebre neix als "Picos de Europa" situat a la serralada Cantàbrica, i flueix del nord-oest al sud-est cap al mar Mediterrani, on acaba formant el Delta de l'Ebre. Al llarg del seu curs, l'Ebre recull les aigües dels rius Segre, Cinca, Aragón, Gállego i Jalón, considerats els seus tributaris principals. En la major part del seu recorregut es caracteritza per un clima mediterrani-continental, excepte a l'àrea del nord dels Pirineus, on hi ha una transició al clima de muntanya, i a les capçaleres, on hi ha un clima atlàntic, mentre que al tram mig trobem un clima semi àrid. La conca de l'Ebre té una mitjana anual de precipitació que varia dels 250 als 2200 mm, amb els valors més alts i més baixos al nord-oest i sud-est, respectivament. Per tant, la mitjana de temperatura anual mostra els registres més baixos a la zona dels Pirineus, i els més alts a l'àrea semiàrida, amb uns mínims entre els 0-12 ºC i uns màxims entre els 7-25ºC. La densitat demogràfica en aquest territori és baixa si es compara a la mitjana de l'Estat Espanyol, amb 33 hab/km^2, i un total de 2.800.000 persones que viuen principalment al tram mitjà del riu.

La conca de l'Ebre és veu afectada per múltiples impactes d'origen industrial, urbà i agrícola (Pujol i Sánchez-Cabeza, 2000; Lacorte et al., 2006; Terrado et al., 2006), encara que el principal ús del sòl sigui l'agricultura, representant fins al 90% de consum d'aigua a causa dels regadius (783.948 ha d'àrea irrigada). El riu està altament regulat per la construcció de més de 190 grans preses que retenen fins al 60% del cabal anual per a la generació d'hidroelectricitat i per a la irrigació (Vericat i Batalla, 2006). D'altra banda, la conca de l'Ebre té un gran valor pel que fa a biodiversitat, ja que un 3,2% és zona protegida, incloent-hi 3 reserves de la biosfera (210,338 ha), 2 parcs nacionals (56,370 ha), 3.500.000 ha incloses a la xarxa Natura 2000 i 15,573 ha incloses al conveni RAMSAR (vegeu www.chebro.es).

El context d'aquesta tesi se situa al tram baix del riu Ebre, concretament als darrers 100 quilòmetres de riu, situat a la part catalana de l'Ebre. Aquesta part del riu té una mitjana anual de cabal natural de 426 $m^3 \cdot s^{-1}$ a Ascó (Confederació Hidrogràfica de l'Ebre, URL:

http: www.chEbro.es), regulat per un complex de tres grans embassaments construïts entre els anys 1940 i 1970: Mequinença, Riba-Roja i Flix, amb una capacitat de 1534 hm^3, 207 hm^3, i 11 hm^3, respectivament. La presència d'aquests embassaments ha canviat la hidrologia, la geomorfologia i l'ecologia del riu, alterant la magnitud, la sincronització i la durada dels cabals (Batalla et al., 2004), la dinàmica del sediment (Vericat & Batalla, 2006, 2007), els règims de temperatura de l'aigua, i la geoquímica (Ibáñez et al., 1995; Sabater et al., 2008; Prats et al., 2010). Per tant, la regulació del riu es considera l'impacte antropogènic principal en el sistema. Durant el segle XX s'ha constatat una tendència a la reducció de la mitjana anual del flux del 29%, atribuïda principalment a l'ús de la irrigació però també a l'evaporació ocorreguda en els embassaments (Ibáñez et al., 1996), que afecta la dinàmica de la falca salina a l'àrea d'estuari.

Si comparem les dades hidrològiques recents amb el context històric, la situació actual escaracteritza per la reducció de grans crescudes a la tardor i hivern, un lleuger augment a la primavera i al començament de l'estiu, degut al desembassament necessari després que es fongui la neu als Pirineus, i per un increment dels cabals mínims a l'estiu (Sánchez & Ibáñez, 2008). Durant els darrers 20 anys, les concentracions de nutrients dissolts han mostrat una tendència a la disminució dels fosfats, atribuïda sobretot a l'augment de les depuradores d'aigües residuals a la conca, encara que els nivells de nitrats hagin estat similars a causa dels usos del sòl amb finalitats agrícoles (Ibáñez et al., 2008). Així doncs, juntament amb la presència de musclo zebrat (*Dreissena polymorpha* [Pallas]) als embassaments i la proliferació de macròfits a la secció del riu, s'ha detectat una forta disminució de la biomassa de fitoplàcton (Sabater et al., 2008; Ibáñez et al., 2008).

Dins dels altres impactes antropogènics en l'àrea d'estudi, la presència d'una central nuclear construïda el 1984 a Ascó, 5 quilòmetres aigües avall de darrer gran embassament, produeix un augment de la temperatura de l'aigua de fins a 3ºC, que pot variar depenent del cabal de l'aigua (Prats et al., 2010). D'altra banda, en vista dels efectes de l'escalfament global (IPCC, 2007), es preveu un augment de 3,2 ºC per al període 2070-2100 a l'àrea de l'Ebre (CHE, 2005), on ja s'han observat alguns efectes sobre la fenologia en espècies d'ocells, insectes i plantes terrestres (Gordo & Sanz 2005). Pel que fa a la contaminació, l'impacte més significatiu al tram baix del riu Ebre és el vessament de més de 300.000 t de residus sòlids provinents de la indústria

electroquímica situats a l'embassament a Flix, i que conté principalment metalls pesants i compostos organoclorats (Grimalt, 2006).

La fàbrica química de Flix va ser fundada el 1899 i ubicada a prop el riu Ebre, a causa de la seva posició estratègica pel que fa a temes d'infraestructures de transport i de proveïment de matèria primera (Sánchez & Visa, 1994). A causa de la construcció de l'embassament de Flix el 1949, els residus industrials i els sediments naturals que prèviament transportava el riu van començar a dipositar-se a la presa. Tot i això, com que les grans avingudes encara eren possibles a causa de la poca capacitat de la presa, només es va acumular una part del sediment i materials contaminats. Quan a finals dels anys seixanta es van construir les preses hidroelèctriques de Riba-Roja i Mequinença aigües amunt de Flix, els sediments naturals hi van quedar retinguts, de manera que a Flix només s'hi van dipositar els productes industrials. La quantitat més gran de sediments contaminats va ser dipositada a partir de l'any 1973 fins al 1984, principalment productes dels fertilitzants del fosfat, fins al moment que el vessament d'aquests productes va ser prohibit. Els contaminants presents en aquests sediments són transportats aigües avall (Gómez-Gutiérrez et al., 2006) i s'han detectat en organismes de la cadena tròfica del tram baix del riu Ebre, des de la font de contaminació fins al delta (Schuhmacher et al., 1993; Ramos et al., 1999; Mañosa et al., 2001; Sánchez-Chardi et al., 2007; Cid et al., 2010; Suárez-Serrano et al., 2010; Barata et al., 2010).

L'àrea d'estudi d'aquesta tesi se situa dins de la part d'aigua dolça del tram baix de l'Ebre, des de la presa de Flix fins a la ciutat de Tortosa. El tram més baix del riu que està sota la influència de la falca salina no s'ha inclòs en aquesta tesi ja que el nostre estudi no se centra en les comunitats d'aigües de transició (tanmateix, vegeu Guillén et al., 1992; Ibáñez et al., 1997 per a una descripció detallada de l'estuari i de les seves característiques hidrològiques). Dins la part del riu estudiada en aquesta tesi, el substrat del sediment es compon principalment de graves, còdols i sorra entre els intersticis (Muñoz & Prat, 1994; Batalla & Vericat, 2009), i al llarg del seu curs s'hi poden trobar diverses illes i braços fluvials que proporcionen una major heterogeneïtat de l'hàbitat. La vegetació ripària cobreix una àrea limitada a cada marge del riu, ja que les terrasses fluvials adjacents han estat modificades per l'agricultura (vegeu Limnos, 1998; Prats et al., 2010). Encara que la vegetació autòctona sigui predominant, amb espècies com el tamariu (*Tamarix* sp.), el pollancre (*Populus nigra* Linnaeus), l'àlber (*Populus alba* L.), el salze (*Salix* sp.), el canyís (*Phragmites australis* [Cav.] Steudel) i la boga (*Typha*

sp.), als llocs més antropitzats la vegetació principal es compon per canya americana (*Arundo donax* L.), una espècie introduïda (Fernández-González, 1990; Molina-Holgado, 2003; Curcó, 2007).

Des de principis dels anys 80, al tram baix del riu Ebre s'han dut a terme estudis d'investigació ecològica i limnològica als embassaments, a la part del riu, a l'estuari i al delta, descrivint l'estructura de les comunitats principals, els processos ecològics i l'avaluació dels impactes principals en l'ecosistema (de Sostoa et al., 1985; Muñoz, 1990; Muñoz & Prat, 1994; Ibáñez et al., 1995; Ibáñez et al., 1996; Limnos, 1998; Mañosa et al., 2001; Ibáñez i Prat, 2003; Val et al., 2003; Benejam et al., 2009; Cid et al., 2008, 2010; Suárez-Serrano et al., 2010, entre molts altres). Pel que fa a la part fluvial del tram baix de l'Ebre, considerat com a objecte d'estudi en aquesta tesi, les descripcions anteriors de la composició de la comunitat s'han realitzat principalment en macroinvertebrats, ictiofauna, i fitoplàncton. En aquesta àrea la comunitat de macroinvertebrats estava dominada pels quironòmids, com ara *Cricotopus* spp. i Oligoquets, encara que també n'eren característics filtradors com *Hydropsyche exocellata* Dufour i l'*Ephoron virgo* (Olivier), col·lectors com *Caenis* spp., i brostejadors com *Theodoxus fluviatilis* L. (Muñoz & Prat, 1994, Limnos, 1997). Avui dia, la comunitat de macroinvertebrats inclou poblacions abundants d'espècies no autòctones, com el musclo zebrat (*D. polymorpha*) i la cloïssa asiàtica (*Corbicula fluminea* [O.F. Müller]) (Jiménez-Muer, 2001; Sabater et al., 2008; Navarro et al., 2006), mentre que les espècies natives com la nàiade *Margaritifera auricularia* (Spengler) es troben en perill i la població disminueix (Altaba, 1990; Araujo & Ramos, 2000). També s'ha detectat un augment massiu de les poblacions de mosca negra (*Simulium erythrocephalum* [De Geer]), lligada a la proliferació massiva de macròfits on habiten i que prova continus problemes de salut pública a la zona degut a les picades.

La comunitat de peixos a l'àrea estudiada està constituïda per una part molt elevada d'espècies no autòctones, com el silur europeu (*Silurus glanis* L.) introduït a final dels anys 60 als embassaments. L'extinció d'espècies anàdromes autòctones, com l'esturió atlàntic (*Acipenser sturio* L.), o el declivi de la saboga (*Alosa fallax* [Lacepéde]) s'ha atribuït principalment a l'impacte de barreres físiques com les preses i assuts, als impactes urbans i industrials, i a la regulació del riu (Sostoa & Lobon-Cervia, 1989; Fernández i Farnós, 1999), encara que dades recents han mostrat una lleugera

recuperació de les poblacions de saboga en aquesta àrea (López et al., 2007). Altres espècies importants a l'Ebre són considerades endèmiques (*Barbus graellsii* Steindachner i *Chondrostoma miegii* Steindachner), vulnerables (*Anguilla anguilla* L.) o amenaçades (*Salaria fluviatilis* Asso) (Doadrio, 2002; Ibáñez & Prat, 2003).

Avui dia, la comunitat de fitoplàncton de l'Ebre aigües avall dels embassaments és poc nombrosa i es compon principalment de diatomees cèntriques i de cloròfits colònials o unicel·lulars, com *Cyclotella* sp., *Stephanodiscus* sp., *Coelastrum microporum* Nägeli, *Pediastrum* sp., *Gymnodinium* sp. i *Peridinium* sp. (Sabater & Muñoz, 1990; Sabater & Klee, 1990; Sabater et al., 2008). A finals dels anys 80, tot i que els embassaments ja existien, les altes concentracions de fosfat dissolt i l'absència de musclo zebrat als embassaments va propiciar una comunitat de fitoplàncton més abundant, encara que la seva composició sigui similar a la d'avui dia. D'altra banda, la comunitat d'algues bentòniques està principalment dominada per *Cladophora* sp. i per una comunitat diversa de diatomees, incloent espècies com l'*Amphora pediculus* (Kützing) Grunow, *Cocconeis placentula* var. lineata Ehrenbergh, *Navicula cryptotenella* Lange-Bertalot, *Nitzschia dissipata* (Kützing) Grunow, *N. palea* (Kützing) W.Smith i *N. incosnpicua* Grunow, entre les més abundants (comunicació personal de Rosa Trobajo).

El valor mitjà de la concentració de clorofil·les en algues bentòniques va ser de 50-250 mg/m^2 el 2005 i 2006 (Sabater et al., 2008). La proliferació de macròfits al tram més inferior del riu durant els darrers 10 anys és també un fet rellevant ja que ha induït canvis ecològics que afecten la salut pública i l'economia a l'àrea (Ibañez et al., 2008; Batalla et al., 2009). Els taxons principals de macròfits trobats a l'Ebre són el *Potamogeton pectinatus* L., *Myriophyllum spicatum* L.. i *Ceratophyllum demersum* L. (Limnos, 1998; Andreu, 2007), en gran part presents en àrees de poc cabal, malgrat que el *P. pectinatus* es pot trobar en gairebé tots els hàbitats.

Aquesta tesi integra els diversos tipus d'impactes i les pressions sofertes per l'ecosistema fluvial del tram baix de l'Ebre, considerant l'efecte sobre els macroinvertebrats aquàtics a diversos nivells d'organització. A la secció següent es presenten els objectius i l'estructura principal de la tesi.

OBJECTIUS

L'objectiu general d'aquest estudi era avaluar els efectes dels principals factors de pressió ambiental sobre l'ecosistema d'aigua dolça del tram baix de riu Ebre mitjançant la comunitat de macroinvertebrats bentònics. Per assolir aquest objectiu es van tenir en compte diversos nivells d'organització (comunitat, població i individu) així com diferents metodologies en funció del tipus de pressió.

Aquesta tesi doctoral es divideix en 5 capítols, que corresponen a 5 articles (2 dels quals ja han estat publicats, un està enviat i els dos restants que seran enviats per publicar properament).

Concretament, cada capítol vol respondre a les preguntes següents:
Capítol 1. La composició taxonòmica i biològica de la comunitat es veu influïda per la distància respecte als embassaments, i pel vessament de sediments contaminats? Hi ha una variabilitat temporal? Les mètriques de la comunitat són condicionades per aquests factors?
Capítol 2. Les condicions de l'hàbitat, com la hidràulica, l'oxigen i la coberta de macròfits determinen la distribució de les comunitats de macroinvertebrats? Quines són les seves preferències hidràuliques? És important la resolució taxonòmica per determinar aquestes preferències? Hi ha alguna resposta biològica a aquests factors ambientals? La hidràulica repercuteix en les mètriques de la comunitat?

Capítol3. Hi ha alguna influència del canvi de temperatura de l'aigua en la producció secundària i el cicle biològic dels insectes aquàtics? Hi ha algun canvi en les poblacions de filtradors autòctons com l'*Ephoron virgo* després de l'establiment d'espècies introduïdes, amb els mateixos hàbits alimentaris (*Corbicula fluminea* i *Dreissena polymorpha*)?

Capítols 4 i 5. Existeix una bioacumulació de contaminants en l'ecosistema fluvial del riu Ebre? Aquests contaminants provenen del vessament de sediments tòxics situats aigües amunt de l'àrea d'estudi? Quins són els patrons de bioacumulació al llarg del creixement i de les etapes del cicle biològic d'espècies sensibles a contaminants com l'*Ephoron virgo*? Hi ha diferències en la bioacumulació entre espècies sensibles (*Ephoron virgo*) i tolerants (*Hydropsyche exocellata*)? Hi ha diferències entre els composts analitzats?

Aquestes preguntes es responen al llarg de cada capítol, i es tracten en global a la discussió de la tesi. Finalment es presenten les conclusions.

DISCUSSIÓ GENERAL

L'objectiu d'aquesta discussió és donar una perspectiva general dels punts principals tractats al llarg de la tesi i justificar-ne les conclusions. Com els impactes antropogènics al tram baix del riu Ebre s'han descrit prèviament a la introducció, aquesta discussió se centra en els dos impactes principals: l'avaluació i els efectes de les alteracions hidrològiques i la importància de la contaminació per metalls pesants i organoclorats (OC), utilitzant els macroinvertebrats bentònics com a bioindicadors. En general, aquesta tesi demostra que l'ecosistema del tram baix del riu Ebre pateix les conseqüències de les alteracions hidrològiques i sofreix un estrès antropogènic important, experimentant una bioacumulació elevada de contaminants en espècies clau que van donar lloc a canvis en l'estructura i la funció de la comunitat.

En els rius altament regulats com l'Ebre, les condicions hidrodinàmiques estan alterades i en conseqüència afecten a la composició i a la diversitat de les comunitats aquàtiques aigües avall dels impactes de manera tan directa com indirecta, incloent la fragmentació l'hàbitat i la seva homogeneïtzació, l'empitjorament de qualitat de l'aigua i la presència d'espècies invasores. En aquest context, l'ús de macroinvertebrats com a indicadors d'alteracions hidrològiques té una llarga tradició en la gestió, incloent estudis de cabals ambientals (Gore, 1978; Gore, 2001; Suren & Jowett, 2006; James & Suren, 2009; Dunbar et al., 2010). Al **Capítols 1** i **2**, es va avaluar la resposta de la comunitat de macroinvertebrats sota diverses situacions hidrològiques i hidràuliques, utilitzant tant la taxonomia (estructura) com els trets biològics (funció).

Al **Capítol 1**, la variació temporal corresponent a diferents condicions hidrològiques va quedar reflectida en major part en la composició de la comunitat i les mètriques funcionals. Una situació prèvia de cabal relativament alt a la primavera va determinar una comunitat amb una diversitat funcional més baixa que no pas a la tardor, mentre que el llarg període de cabals estables i relativament baixos abans de la tardor van propiciar una diversitat funcional més alta. Tanmateix, per a una millor interpretació de la resposta funcional a la variabilitat del cabal, es requereixen estudis que utilitzin mesures

físiques directes, com ara la velocitat de l'aigua (Statzner & Bêche, 2010). Aquesta idea es va tractar al **Capítol 2**, on es va demostrar que la major part de les categories dels trets biològics considerats en l'estudi van respondre (positivament o negativament) a canvis en la velocitat de l'aigua, nombre de Reynolds i nombre de Froude. Diversos estudis han investigat la resposta de la composició dels trets biològics a les condicions hidràuliques (Lamoroux et al., 2004; De Crespin et al., 2002; Snook & Milner, 2002; Mérigoux & Dolédec, 2004; Tomanova & Usseglio-Polatera, 2007; Horrigan & Baird, 2008), demostrant que els trets són un filtre primari que determinen quina espècie pot sobreviure i reproduir-se sota certes condicions ambientals (River Habitat Templet; Southwood, 1988; Poff &Ward, 1990; Townsend & Hildrew, 1994), i que l'hàbitat físic és un dels factors més importants per determinar l' estructura la comunitat d'invertebrats aquàtics en cursos fluvials.

De la mateixa manera que en el cas dels trets biològics, un bon coneixement del nínxol ecològic que ocupa cada espècie a partir de mesures hidràuliques directes proporcionaria respostes més específiques de la comunitat a les variacions de corrent de l'aigua. Així, els requisits hidràulics de les espècies i la resposta dels seus trets biològics podrien proporcionar una base per al seu ús en la gestió de cabals ambientals (Gore et al., 2001), sobretot si tenim en compte que les respostes funcionals a nivell de microhàbitat poden predir les respostes a escala de tram (Lamoroux et al., 2004) i que les espècies que presenten una elevada marginalitat pel seu hàbitat serien les més sensibles a les alteracions hidrològiques (Dolédec et al., 2007). D'altra banda, com més alta és la resolució taxonòmica, més exacta serà la nostra interpretació de la seva resposta, atès que es van obtenir diverses respostes a les condicions hidràuliques per al mateix gènere (ex. *Cricotopus (Cricotopus*) *trifascia* i *C.(C.) bicinctus*) (**Capítol 2**).

Els **Capítols 1** i **2** representen un dels pocs estudis que comparen al mateix temps canvis estructurals i funcionals de la comunitat de macroinvertebrats en grans rius, encara que aquest esforç s'hagi fet prèviament prenent en consideració grans bases de dades a grans escales espacials a Europa i Amèrica del Nord (Bady et al., 2005; Bonada et al., 2007; Bêche & Statzner, 2009; Péru & Dolédec, 2010).

En el context de canvi climàtic, s'ha predit un augment de les temperatures i una reducció de les precipitacions en els climes mediterranis (IPCC, 2007), i els impactes en els recursos hídrics del clima de la conca de l'Ebre ja han estat objecte d'estudi (CHE,

2005). Aquesta situació, així com els projectes de transvassaments i concessions d'aigua a l'Ebre a causa de canvis en els usos del sòl (agricultura), pot comportar problemes de gestió en un moment on s'estan considerant propostes referents a cabals ecològics a l'Ebre (Sánchez & Ibáñez, 2008) i la Directiva Marc de l'Aigua (Comissió Europea, 2000) ha de ser implementada. De moment, en resposta a un augment de la temperatura s'han detectat canvis en la fenologia dels insectes terrestres, els ocells i les plantes (Gordo & Sanz, 2005), i també en els insectes aquàtics (**Capítol 3** d'aquesta tesi).

L'alteració del règim de cabals afecta als peixos i a la composició de la comunitat de macroinvertebrats i augmenta la introducció d'espècies no autòctones (Poff et al., 2010). Així, tal i com es mostra als **Capítols 1** i **2**, les poblacions d'espècies introduïdes com ara la *Corbicula fluminea* eren dominants, fet que es podria explicar per la seva alta capacitat reproductiva i els seus baixos requisits hidràulics, ja que presenten un rang molt ampli d'hàbitat potencial. Tanmateix, malgrat l'alta abundància d'aquest filtrador al tram més baix del riu Ebre, i l'alta presència de musclo zebrat (*D. polymorpha*) als embassaments, els resultats de la producció secundària després de 18 anys en poblacions de l'espècie indicadora *Ephoron virgo* van mostrar fins i tot valors més alts que els de 1987 (**Capítol 3**).

Això podria ser a causa de la disminució dramàtica de les poblacions autòctones de nàiades com ara la *Margaritifera auricularia* o l'*Unio elungatulus* C. Pfeiffer (Ramos, 1998; Araujo & Ramos, 2000), no detectats en cap de les mostres que es van recollir durant aquest estudi, atès que l'acció filtradora de la *Corbicula* hauria pogut substituir la competència que exercien anteriorment les nàiades.

Als **Capítols 4** i **5** es va investigar la bioacumulació de metalls pesats i de compostos organoclorats a nivell individual i de població en macroinvertebrats bentònics aigües avall del vesssament de sediments tòxics situats a l'embassament de Flix. Els organismes d'estudi van ser una espècie tolerant (*H. exocellata*) i una de sensible (*E. virgo*) que van presentar diferents nivells de bioacumulació de metalls pesats. Si es té en consideració que ambdós espècies presenten trets biològics similars pel que fa als hàbits de respiració i d'alimentació, tots dos són filtradors i respiren per les brànquies (Tachet et al., 2000), es va considerar que tindrien uns nivells d'exposició a contaminants similars, tant mitjançant la ingestió de partícules en suspensió, o mitjançant la respiració pel contacte directe amb contaminants dissolts.

Malgrat que múltiples factors d'estrès poden actuar al mateix temps al tram baix del riu Ebre, els alts nivells de bioacumulació detectats en l'*H. exocellata* (**Capítol 4**) i l'absència o baixa densitat d'espècies considerades sensibles als contaminants (*E. virgo*) en els primers quilòmetres aigües avall del vessament de sediments tòxics (**Capítol 1**), indiquen que els canvis a nivell de població (abundància relativa) podien ser principalment atribuïts a la contaminació química. A causa dels diferents trets ecofisiològics de cada espècie que s'atribueixen a un diferent origen filogenètic (Buchwalter et al., 2008), aquelles espècies amb trets biològics similars però genèticament distants entre elles poden presentar diferencies en la seva capacitat per mantenir poblacions estables en cursos fluvials contaminats. La més elevada bioacumulació de metalls trobada en l'*E.virgo* comparat amb l'*H. exocellata* al **Capítol 4** va evidenciar aquests trets ecofisiològics. L'*H exocellata*. pot detoxificar i eliminar fàcilment els contaminants (Cain et al., 2004; Buchwalter et al., 2008) i les seves poblacions no es van veure afectades per la contaminació, mentre les poblacions l'*E. virgo* no van començar a ser mitjanament abundants a partir dels primers 21 km aigües avall de Flix i només considerablement abundants en la zona de mostreig situada a 65 km de la font de la contaminació (**Capítol 3**). D'aquesta manera, l'estructura de la comunitat variava a mesura que la distància des dels sediments de Flix i de les preses augmentava. Aquests canvis a nivell de comunitat s'han observat en ecosistemes aquàtics que pateixen l'impacte de contaminants tòxics (Liess et al., 1996; Hutchens et al., 1998; Clements et al., 2000; De Lange et al., 2004; Cain et al., 2004), amb l'especial rellevància dels Efemeròpters, atès que són generalment una de les primeres poblacions que mostren disminucions significatives en la seva abundància i riquesa en llocs impactats (Edsall et al., 1991; Clements et al.,, 2000; Maret et al.,, 2003). D'altra banda, els efectes indirectes de la contaminació per metalls o pesticides sobre poblacions sensibles poden augmentar-ne la taxa de predació (Clements, 1999; Schultz & Dabrowski, 2001), atès que els efectes subletals poden portar a canvis del comportament animal (e.g., locomoció reduïda). Això podria ser possible a l'Ebre, ja que que les nimfes de l'efemeròpter *E. virgo* que vivien aigües amunt de Flix (Saragossa) van presentar una mobilitat més alta que els que es van observar aigües avall (observació personal). Considerant tot això, l'*E. virgo* ha servit com a un bon bioindicador de la contaminació atès que és sensible als contaminants tòxics (De Haas et al.,, 2002) i per tant es considera una espècie d'especial rellevància per la protecció dels ecosistemes fluvials en la seva àrea de la distribució (Klok & Kraak, 2008), on s'inclou el riu Ebre i

la major part dels grans rius europeus. Més enllà de la distribució espaial de la contaminació, els canvis temporals en l'ecosistema fluvial també poden determinar el grau d'exposició als contaminants per la fauna aquàtica (Chapman et al., 2003). Per exemple, al **Capítol 1** les mètriques basades en la taxonomia (incloent la riquesa, els índexs de diversitat i l'índex biòtic IBMWP) van presentar valors més alts a mesura que augmentava la distància des del focus de contaminació, però només a la primavera. Aquesta resposta més elevada a la primavera era a causa d'aquestes espècies sensibles d'Eferemeròpters com ara l'*E. virgo* o el *Choroterpes pictetii*, que estan només presents com a nimfes durant la primavera i l'estiu degut al seu cicle biològic univoltí (un cicle biològic per any). Per tant, el cicle biològic de les espècies clau ha de ser considerat per al diagnosi de la contaminació a escala espacial i temporal. A més, l'Ebre, encara que és un riu altament regulat, els cabals elevats de principi de primavera podrien comportar una exposició més elevada als contaminants a causa de l'alliberament de sediments tòxics procedents de la presa de Flix i d'altres àrees de deposició aigües avall.

D'altra banda, les mètriques basades en els trets biològics (riquesa de trets, diversitat funcional) van mostrar només canvis lleus en la comunitat al llarg del gradient de contaminació. Això va mostrar la limitació per establir una causa-efecte de les respostes a nivell de comunitat pel que fa a les mètriques funcionals basades només en els trets biològics (e.g., mida màxima, hàbits alimentaris), atès que no integra els diversos trets ecofisiològics de cada espècie. Tanmateix, l'ús de prediccions *a priori* de les categories seleccionades de cada tret basades en respostes previstes als tipus específics podrien millorar-ne la interpretació (Dolédec & Statzner, 2008). A l'Ebre, atès que els contaminants principals van ser incorporats en major part mitjançant la ingestió de la matèria particulada en suspensió (**Capítol 4**), s'esperaven proporcions més baixes de filtradors en les zones més pròximes a la font de la contaminació. Això es va observar al **Capítol 1**, quan es van utilitzar els trets biològics per detectar canvis funcionals longitudinals des dels embassaments principals i de la font de la contaminació de metalls i d'organoclorats. Fins al moment, només dos articles recents han relacionat els trets biològics d'invertebrats aquàtics amb l'impacte de substàncies tòxiques en els rius (Dolédec & Statzner, 2008; Archaimbault et al., 2010), fet que fa encara difícil interpretar la resposta dels trets biològics a aquest tipus d'impacte. D'altra banda, en la major part dels rius afectats per substàncies tòxiques altres tipus d'impactes poden actuar simultàniament, el que ho dificulta encara més.

En ecosistemes aquàtics contaminats, la biologia i el cicle biològic dels insectes aquàtics han de ser considerats per avaluar el risc de transferència de contaminants a nivells tròfics superiors (Corkum et al., 1997; Smits et al., 2005; Bartrons et al., 2007). Per aquesta raó, als **Capítols 4** i **5** la transferència de metalls i de compostos organoclorats va ser avaluada a través del cicle vital considerant l'*E. virgo* com a model. Segons el que s'ha estudiat en el **Capítol 3**, el cicle vital univoltí i sincronitzat d'aquesta espècie va donar l'oportunitat d'estudiar la transferència de contaminants durant el cicle vital en l'hàbitat natural. Les nimfes de l'*E. virgo* eclosionen a l'abril i tenen un desenvolupament ràpid fins l'època d'emergències dels adults i reproducció a finals de juliol, quan els ous es dipositen al riu i passen la tardor i l'hivern en diapausa (Kureck & Fontes, 1990). A causa de l'alta producció secundària d'aquesta espècie a l'Ebre (amb una producció anual de 950 mg pes sec/m^2/any) i a les seves emergències massives durant l'estiu, l'*E. virgo* és font d'aliment abundant per als peixos i els ocells insectívors. Atès que les concentracions bioacumulades de metalls i compostos organoclorats en insectes aquàtics varia amb el creixement i les diferents etapes del cicle vital (Smock, 1983; Caín et al.,, 1992; Standley et al.,, 1994; Bartrons et al.,, 2007) i que s'han descrit diversos models de bioacumulació per a diversos compostos (Smock, 1983), els **Capítols 4** i **5** van demostrar que els contaminants més persistents com l'Hg, el Cd, PCBs o DDEs van ser transferits en quantitats elevades als adults emergents i als ous, encara que aquests últims presentessin concentracions molt més baixes i en el cas del Cd la transferència als ous fos mínima.

Les concentracions més baixes en ous comparades amb les nimfes i els adults en la majoria dels contaminants analitzats podrien ser un factor clau per explicar l'abundància poblacional de l'*E. virgo* en els llocs més allunyats aigües avall del vessament de sediments tòxics. Al existir problemes de bioacumulació lluny de la font de contaminació, les nimfes podrien patir efectes subletals que afectarien a la reproducció (Conley et al., 2009). Tanmateix, no semblava ser el cas del nostre estudi a causa les grans quantitats d'adults emergents amb proporcions elevades de femelles (**Capítol 3**). Al **Capítol 5** s'explica que la baixa transferència materna de OCs als ous podria estar lligada al contingut del lípids i a la seva composició, als mecanismes del transport de lípids i a l'estructura de la closca de l'ou. Aquests factors podrien fer possible que la transferència de contaminants a la generació següent fos més baixa, encara que l'efecte

sobre l'èxit d'eclosió o sobre la supervivència de les nimfes acabades d'eclosionar no es va arribar a estudiar.

Els diversos patrons de bioacumulació durant el creixement de les nimfes d'*E. virgo* van variar depenent del compost analitzat. Aquestes diferències es poden relacionar amb la mida de la partícula ingerida, les preferències hidràuliques en cada un estadi de creixement, i amb la quantitat de sediments contaminats alliberats des de la presa de Flix depenent dels cabals. Una de les raons per explicar la concentració més baixa de contaminants en nimfes més grans recollides a principis d'agost podria ser que al canviar les seves preferències hidràuliques es traslladen a les àrees de lents per emergir i per tant reflecteixen els contaminants disponibles en aquests habitats, on hi pot haver una exposició més baixa degut al menor transport de partícules en suspensió. Per tant, les preferències hidràuliques dels macroinvertebrats i el règim de cabal del riu són determinants per al coneixement de la dinàmica de la bioacumulació en l'hàbitat natural. Al **Capítol 2**, les espècies que ocupaven àrees amb elevades velocitats del corrent eren sobretot insectes filtradors com l'*H. exocellata* o omnívors com els Orthocladiinae, que podrien ser indicadors de la càrrega de contaminants presents en el flux d'aigua. D'altra banda, espècies que ocupaven àrees de lents com ara col·lectors (e.g., *Caenis luctuosa*) podria reflectir la biodisponibilitat de contaminants en àrees de deposició de sediments en e riu. Aquest punt de vista podia ser útil entendre els patrons de contaminació de la comunitat de macroinvertebrats a escala de mesohàbitat.

A més, atès que s'ha vist que la bioacumulació de contaminants en filtradors marins és dependent de la temperatura (e.g., Odin et al., 1997; Loayza-Muro & Elías-Letts, 2007), si la temperatura global augmenta d'acord amb les previsions (IPCC, 2007), la bioacumulació podria augmentar a causa de taxes de filtració més elevades i de canvis en la química i física dels contaminants (Mubiana & Blust, 2007). Així, a part d'alteracions en la fenologia de les espècies (e.g., avançament del cicle biològic de l'*E. virgo* gairebé d'un mes el 2005 comparats amb el 1987, **Capítol 3**), altres canvis com uns nivells més elevats de bioacumulació amb les corresponents respostes toxicològiques poden ocórrer amb un augment de la temperatura.

Per altra banda, els resultats d'aquesta tesi demostren que l'exposició a metalls i compostos organoclorats van ser originats per la contaminació històrica dels sediments tòxics de Flix, que afecta en gran mesura la fauna aquàtica, reflectit sobretot en espècies sensibles i en els canvis funcionals i estructurals de la comunitat de macroinvertebrats.

Llavors, una recuperació de les espècies sensibles com l'*E. virgo* en els llocs on actualment la contaminació per metalls i compostos organoclorats és elevada seria un indicatiu d'una millora ecològica. Atès que s'està executant un pla de restauració que consisteix en l'extracció dels sediments tòxics de Flix (Resolución de la Confederación Hidrogràfica del Ebro, 2006), i la Directiva Europea 2006/11/CE referent a substàncies perilloses en ecosistemes aquàtics ha de ser implementada, en un futur pròxim es podran observar canvis positius en poblacions d'espècies sensibles i reduccions dels nivells de bioacumulació en l'ecosistema del tram baix de l'Ebre.

Com que molts altres factors ambientals són també determinants per a la composició de la comunitat de macroinvertebrats (règim de cabals, oxigen, disponibilitat d'aliment, presència de macròfits i altres aspectes de l'hàbitat) és difícil separar els altres tipus d'impacte de la contaminació de metalls i organoclorats. Tanmateix, aquesta tesi proporciona una prova evident de la resposta ecològica als impactes antropogènics que està patint el tram baix del riu Ebre actualment, posant en evidència canvis en els invertebrats aquàtics a diversos nivells d'organització, incloent comunitats, poblacions i individus.

CONCLUSIONS

Les conclusions principals de la tesi, i que contesten a les qüestions plantejades en els objectius són les següents:

1. A nivell de comunitat, l'anàlisi basat en la taxonomia va mostrar que la composició de la comunitat canviava a mesura que la distància des dels principals impactes antropogènics incrementava (embassaments i sediments contaminats). Aquests canvis van influir en les mètriques basades en la taxonomia, amb valors més alts als llocs situats més aigües avall a causa de la presència d'espècies sensible a la contaminació química.
2. La variabilitat temporal en relació a diferents condicions hidrològiques i estacionals va ser reflectida principalment en l'anàlisi de tret biològics (composició i mètriques funcionals), mentre que utilitzant les mètriques taxonòmiques es van detectar canvis mínims.
3. D'entre totes les variables mesurades a escala de mesohàbitat, les mesures directes com la velocitat de l'aigua i el nombre de Froude i de Reynolds van explicar la major

part de la variabilitat de la distribució de macroinvertebrats bentònics i dels seus trets biològics. Tanmateix, la cobertura de macròfits present en el bentos i l'oxigen dissolt intersticial no van ser determinants.

4. En mesurar el nínxol ecològic de cada espècie de la comunitat, els taxons amb una alta marginalitat van mostrar una menor disponibilitat d'hàbitat (bé típics de corrents ràpids o de lents), mentre que altres espècies presentaven una elevada tolerància les diferents condicions de l'hàbitat

5. La resolució taxonòmica va resultar important per obtenir interpretacions acurades de les preferències de la comunitat al llarg del gradient hidràulic, donant rellevància al al grup dels Chironomidae, que van mostrar preferències d'hàbitat diferents dins de la mateixa subfamília, tribu o gènere. Per tant, la resolució taxonòmica va influir la resposta de les mètriques de la comunitat en resposta a les condicions hidràuliques.

6. La major part dels trets funcionals dels macroinvertebrates (trets biològics) van respondre positivament o negativament a les condicions hidràuliques (e.g. alimentació, locomoció) com a resultat d'una adaptació a les condicions ambientals, mentre que altres trets relacionats amb el cicle vital podrien reflectir adaptacions als esdeveniments del règim de cabals o a les interaccions entre les espècies.

7. A nivell poblacional/ individual, un augment de 2ºC de la temperatura mitjana diària de l'aire durant el període del creixement de l'*E. virgo* al 2005 comparat amb el 1987, juntament amb un valor més alt dels graus-dia acumulats el 2005, semblen ser la raó principal per un avançament de 3 setmanes del cicle biològic d'aquesta espècie model.

8. Les estimacions de producció secundària de l'*E. virgo* després de l'establiment d'espècies introduïdes amb els mateixos hàbits alimentaris (*Dreissena polymorpha* i *Corbicula fluminea*) van mostrar valors més alts el 2005 que el 1987, probablement a causa de la gran disminució poblacional de nàiades autòctones,l'activitat filtradora de les quals podria haver estar substituïda progressivament per aquestes espècies no-autòctones.

9. La cadena tròfica del tram baix del riu Ebre presenta problemes importants pel que fa a la bioacumulació de les substàncies tòxiques originades principalment de l'alliberament de sediments procedents dels sediments contaminats de Flix. Una gran varietat de contaminants, incloent-hi els metalls pesants i compostos organoclorats, van ser bioacumulats a elevades concentracions per poblacions de macroinvertebrats filtradors aigües avall de la font de contaminació.

10. Diferents patrons de la bioacumulació van ser observats al llarg del creixemeent del de l'*E. virgo* i al llarg de les etapes del seu cicle biològic. Les nimfes i els adults emergents van presentar les concentracions més altes de metalls i de compostos organoclorats, representant un risc elevat pel que fa a la seva transferència a nivells tròfics superiors. La transferència materna de contaminants als ous es va donar, encara que es va detectar a nivells molt més baixos comparat amb concentracions en femelles adultes.

11. Els diversos contaminants analitzats van presentar differents patrons de bioacumulació, atribuïts principalment a la seva biodisponibilitat, que pot variar depenent del cabal del riu, i de l'afinitat de cada compost pels materials orgànics.

12. Es van observar diferències interespecífiques en la bioacumulació, amb nivells més alts en espècies sensibles com l'*E. virgo* i més baixos en espècies tolerants com l'*H. exocellata*, degut principalment als seus trets ecofisiològics.

En general, la resposta de la comunitat als diversos tipus d'estrès ambiental pot ser complexa, i encara més si es té en compte la contaminació per metalls pesants i composts organoclorats combinats amb alteracions hidrològiques. Tanmateix, ja que només aquelles espècies relativament sensibles a la contaminació química contribueixen a la detecció de canvis a nivell de comunitat, en aquest estudi s'ha demostrat que l'*E. virgo* és un bon bioindicador del risc ecològic causat per contaminants tòxics als grans rius com l'Ebre. D'altra banda, aquest organisme ha estat prèviament utilitzat en assajos de laboratori, incrementant la seva aplicabilitat a altres nivells de l'organització (ex., ús de biomarcadors).

D'altra banda, el coneixement de la resposta de la comunitat de macroinvertebrats bentònics i de la seva estructura funcional a les condicions hidràuliques pot tenir aplicabilitat guiar la gestió de cabals ecològics al tram baix del riu Ebre, proporcionant la informació necessària de cada espècie i que pot ser la base per a l'ús dels models de l'hàbitat utilitzant els macroinvertebrats aquàtics.

BIBLIOGRAFIA

Veure la bibliografia de la introducció i la discussió general en anglès.

Director's report

Report of the directors of the Ph.D. Thesis in reference to its derived publications and the student's contribution to them

Dr. Carles Ibáñez Martí, Director of Aquatic Ecosystems, Institute of Research and Technology, Food and Agriculture (IRTA), Sant Carles de la Ràpita, Catalonia, Spain, as supervisor

and,

Dr. Narcís Prat i Fornells, Professor of the Department of Ecology (University of Barcelona), member of the consolidated research group F.E.M. (Freshwater Ecology and Management), as co-supervisor

of the Ph.D. Thesis authored by Mrs. Núria Cid Puey and entitled *Ecology of the benthic macroinvertebrates in the lower Ebro River: community characterization, population dynamics and bioaccumulation of pollutants in response to environmental factors*

INFORM

That the results and conclusions achieved in the research developed by Mrs. Núria Cid Puey as part of her Ph.D.Thesis have been organised in 5 chapters which correspond to 2 publications and 3 manuscripts, either submitted or ready to be submitted to the corresponding journals. Following, the list of publications and manuscripts is shown, indicating the journal impact factor IF (according to SCI of ISI Web of Knowledge, Journal Citation Report-2009) as well as the median impact factor of the main subject categories and the position of the journal within the corresponding category.

1- Cid, N., Ibánez, C., Andreu, R., and Prat, N. Comparative use of taxonomy and trait-based approaches in macroinvertebrates: importance of flow regulation and sediment pollution in: the lower Ebro River. *Hydrobiologia* (to be submitted). Impact Factor: 1.754 (ISI Journal Citation Report 2009). This journal is reported in the subject 'Marine and Freshwater Biology' and its median impact factor is 1.352, being the 27th of 88 journals in the subject area.

2- Cid, N., Ibánez, C., Andreu, R., Collado, R. and Prat, N. Hydraulic conditions as a key factor for benthic macroinvertebrate assemblages, biological trait response and diversity in a large regulated river**.** *Freshwater Biology* (submitted). Impact Factor: 2.861 (ISI Journal Citation Report 2009). This journal is reported in the subject 'Marine and Freshwater Biology' and its median impact factor is 1.352, being the 8th of 88 journals in the subject area.

3- Cid, N., Ibánez, C., and Prat, N. 2008. Life history and production of the burrowing mayfly *Ephoron virgo* (Olivier, 1791) (Ephemeroptera: Polymitarcyidae) in the lower Ebro River: a comparison after 18 years. *Aquatic insects* 30 (3):163–178. Impact Factor: 0.311 (ISI Journal Citation Report 2009). This journal is reported in the subject 'Entomology' and its median impact factor is 0.891, being the 69th of 74 journals in the area.

4- Cid, N., Ibánez, C., Palanques, A., and Prat, N. 2010. Patterns of metal bioaccumulation in two filter-feeding macroinvertebrates: Exposure distribution, inter-species differences and variability across developmental stages. *Science of the Total Environment* 408 (3): 2795–2806. Impact Factor: 2.905 (ISI Journal Citation Report 2009). This journal is reported in the subject 'Environmental Sciences' and its median impact factor is 1.473, being the 31th of 180 journals in the area.

5- Cid, N., Lourencetti, C., Ibánez, C., Prat, N., and Grimalt, J. O. Organochlorine bioaccumulation in the filter-feeding mayfly *Ephoron virgo* during life cycle in a site with chronic pollution. *Environmental Science and Technology* (to be submitted). Impact Factor: 4.630 (ISI Journal Citation Report 2009). This journal is reported in the subject categories 'Engineering, Environmental' and 'Environmental Sciences'. In the first category, its median impact factor is 1.389, being the 2nd of 42 journals in the area. In the second category, its median impact factor is 1.473, being the 7th of 180 journals in the thematic area.

and CERTIFY

that Mrs. Núria Cid Puey contribution has been very active, as it is demonstrated by his first coauthoring of all the manuscripts that conform this Ph.D. Thesis. Concretely, his participation included the following tasks:

– Definition of the objectives and focus of the research and its derived manuscripts.

–Experimental design and field work, including water, periphyton and macroinvertebrate samples collection, and in situ physico-chemical measurements.

– Heavy metal analysis.

– Periphytic Algal Biomass (Chlorophyll a) and Periphytic Particulate Organic Biomass analysis.

– Sorting, counting and identification of macroinvertebrate families and species.

– Preparation and identification at species level of Chironomidae larvae.

– Biomass and secondary production calculations for keystone species.

– Results compilation and data analysis and interpretation.

– Tables and Figures design and preparation.

– Main writting of the manuscripts, and contact person for the reviewing and editing process.

Finally, we certify that any of the coauthors of the manuscripts detailed above has used, neither is going to use, implicitely or explicitely, the information produced and presented with the purpose of elaborating another Ph.D. Thesis.

Barcelona, July 23th 2010

Dr. Carles Ibáñez i Martí Dr. Narcís Prat i Fornells

General introduction

Ecosystems benefit people well-being by its contribution to material provisioning, supporting and regulation of environmental processes and cultural values (Figure 1). In this context, rivers provide water supply for the basic human activities and profits, namely domestic needs, agriculture, industry, power generation, transportation, fishing and other recreational activities (Allan & Castillo, 2008). As a result of the intensification of these human activities, the deterioration of freshwater ecosystems is been particularly notable during the last 50 years, mainly due to the alteration of natural processes, the overexploitation of resources and to pollution. Large rivers, specially the lower courses, receive a wide range of human impacts where hydrological and geomorphological alterations, land-uses, contaminants and the introduction of alien species have been considered the main pressures (Petts et al., 1993; Poff et al., 2007; Klok & Kraak, 2008; Strayer et al., 2008). Consequently, threats as habitat alteration and loss, change in trophic structure and decline in water quality have lead to changes in ecosystem processes and to biological impairment. Moreover, the predictions of climate change (IPC, 2007) with a reduction of precipitations and a temperature increase depending on the region, together with the projected water withdrawals will exacerbate the level of stress. For instance, the Iberian Peninsula has been included within one of the most threatened areas regarding water stress, pollution and erosion in the near future (Tockner et al., 2009).

Fauna inhabiting freshwater habitats (considering invertebrates, amphibians, fish and birds) represents a species richness value of more than 82.500, where aquatic invertebrates are the most diverse group (see Tockner et al., 2009). However, freshwater fauna showed the highest biodiversity decline compared to terrestrial and marine ecosystems during the last 40 years (Millenium Assessment, 2005). As stress increases,

tolerant and generalist species become dominant and sensitive species become vulnerable or extinct, resulting in a homogenization of the community and loss of diversity and ecosystem functions. These functional loss reflected in the impoverishment of the freshwater communities can have important implications for the ecosystem services, not only influencing rivers but also the estuarine and marine ecosystem. In order to protect the aquatic resources, European environmental legislation developed the EU Water Framework Directive (European Commission, 2000), which consider the ecological integrity of biological communities. The key purposes of this European Directive were reflected in its Article 1, and, according to this legislation, a good ecological status has to be achieved for every European Union state member by 2015.

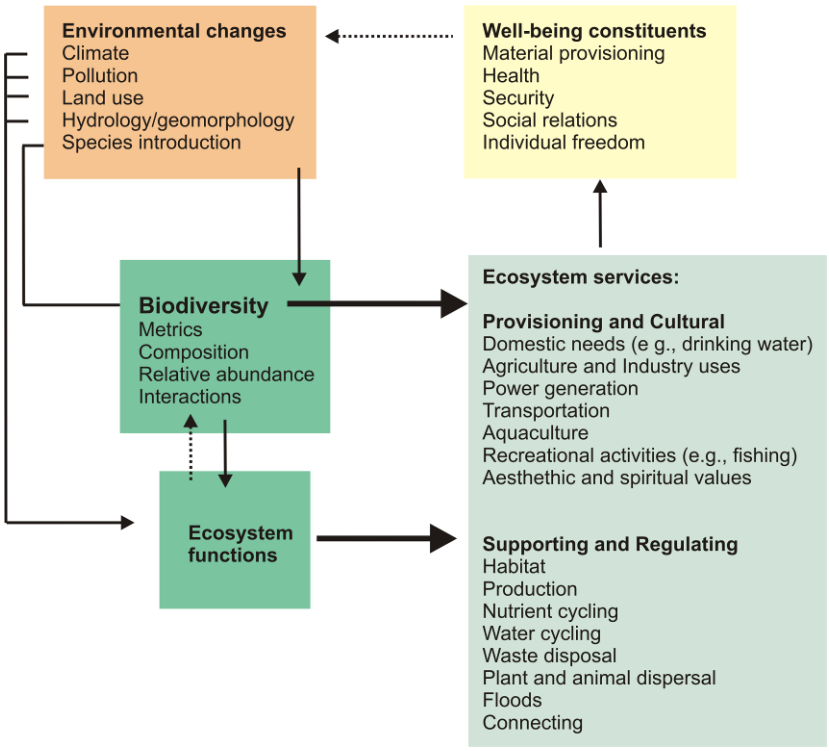


Figure 1. Relationships among Biodiversity, Ecosystem Services, and Human Well-being (Modified from Millennium Ecosystem Assessment: http://www.millenniumassessment.org/en/Synthesis.aspx and adapted to freshwater ecosystems).

Macroinvertebrates have been widely applied in the bioassessment of streams and rivers as bioindicatos because they play a key role in aquatic ecosystems and respond considerably to environmental stressors and disturbances (Rosenberg & Resh, 1993).

Their high biodiversity and biomass contribution into the aquatic foodweb made them organisms of study for the evaluation of pollution status of waters since the beginning of the 20th century until nowadays (Bonada et al., 2006), when they have been integrated in the environmental policies as one of the most important organism groups to define their ecological integrity (European Commission, 2000). By considering these organisms, different approaches have been developed according to the focus of the study and the level of organization. At community level, the taxonomic composition of macroinvertebrates has been the main biomonitoring approach by using multimetric biotic indices based on species tolerance to pollution (Bonada et al., 2004; Bonada et al., 2006) and standarized protocols (e. g. RIVPACS, GUADALMED). Ultimately, the use of several biological invertebrate traits (e.g. body size, reproduction, dispersal) has been considered as an alternative or complimentary tool, assuming that changes in trait composition have consequences on ecosystem functioning (Dolédec et al., 1999; Statzner et al., 2005; Statzner & Beche, 2010). This multitrait approach is based on the concepts of the River Habitat Templet (Townsend & Hildrew, 1994) which a priori predicts the species trait response to natural environmental disturbance, and set the basis to predict anthropogenic impacts in freshwaters (Bonada et al., 2006; Henle et al., 2006). Although studies considering population or individual level are not as abundant as those at community level (Bonada et al., 2005), they increased during the last 10 years, mainly regarding bioaccumulation of contaminants or ecotoxicology (Hare et al., 2001; Barata et al., 2005; De Lange et al., 2005; Cain et al., 2006; Buchwalter et al., 2007) and morphological abnormalities in response to pollutants (Diggins & Steward, 1998; Bonada et al., 2005; Skinner & Bennet, 2007). However, many of these methods observing responses at organism level do not result in changes in the community. For instance, mentum deformities in tolerant *Chironomus riparius* Meigen or the high levels of bioaccumulation in *Hydropsyche* spp. did not change their abundances at highly polluted sites (Cain et al., 2004; De Haas et al., 2005). Despite tolerant species do not contribute to changes in the community, this fact gives them the ability to act as ecosystem sentinels and detect pollution levels and gradients at large spatial scales. Thus, because the responses to stressors as pollutants observed at community level might be a result of effects on relatively sensitive species (e.g. *Ephoron virgo* [Olivier], see Klook et al., 2008), methods at population and individual level in human impacted areas are crucial for these sensitive species. In general, the combined use of a tolerant and sensitive species could provide a more integrative assessment tool (Adams &

Greeley, 2000) since the detection of responses at population level of key species can predict changes at community or even at ecosystem level.

According to these considerations, the use of different bioassessment approaches (community composition and metrics, population dynamics and bioaccumulation) depending on the human impact considered (e.g. flow alterations, pollution, temperature changes, presence of alien species) were tested in the present thesis in the context of the lower Ebro River.

Study context: the lower Ebro River

The Ebro River is located in the NE Iberian Peninsula and has a drainage basin of 85,362 km^2 with a length of 910 km, being the largest river in Spain in terms of water discharge (11.982 hm^3/year at the lowermost part, see www.chebro.es), one of the most important rivers of the Mediterranean and one of the largest catchments in Europe (Tockner et al., 2009). The source of the Ebro River starts at "Picos de Europa" located in the Cantabria mountain range, and flows from north-west to south-east to the Mediterranean Sea, where it ends forming the Ebro Delta. Along its course, the Ebro receives the waters of the Segre, Cinca, Aragón, Gállego and Jalón rivers, considered its main tributaries. A Mediterranean-continental climate is present for the most part, except in the Pyrenees area in the North where there is a transition to mountain climate, in the headwaters where there is an Atlantic climate, and in the middle part where there is a semi-arid climate. The Ebro basin has a mean annual precipitation ranging from 250 a 2200 mm with the highest and lowest values in the North-West and South-East, respectively (Figure 2). Accordingly, the mean annual temperatures are the lowest in the Pyrenees and the highest in the semi-arid area, registering a minimum between 0-12 ºC and a maximum between 7-25 ºC.

The population density in this territory is low compared to the Spanish mean, with 33 people/Km^2 and a total of 2,800,000 people mainly inhabiting the middle course of the river. The Ebro basin is affected by multiple impacts with industrial, urban and agricultural origins (Pujol & Sánchez-Cabeza, 2000; Lacorte et al., 2006; Terrado et al., 2006), although the main land use is agriculture, which represents up to the 90 % of water consumption in the basin due to irrigation (irrigated area of 783.948 ha). The river is strongly regulated by the construction of more than 190 large dams which impound up to the 60% of the mean annual runoff for hydropower generation and irrigation

purposes (Vericat & Batalla, 2006). On the other hand, the Ebro basin has an important biodiversity value, since the 3.2% of the catchment area is protected, including 3 biosphere reserves (210.338 ha), 2 national parks (56.370 ha), 3.500.000 ha included in the Natura 2000 Network and 15.573 ha included in the RAMSAR convention (see www.chebro.es).

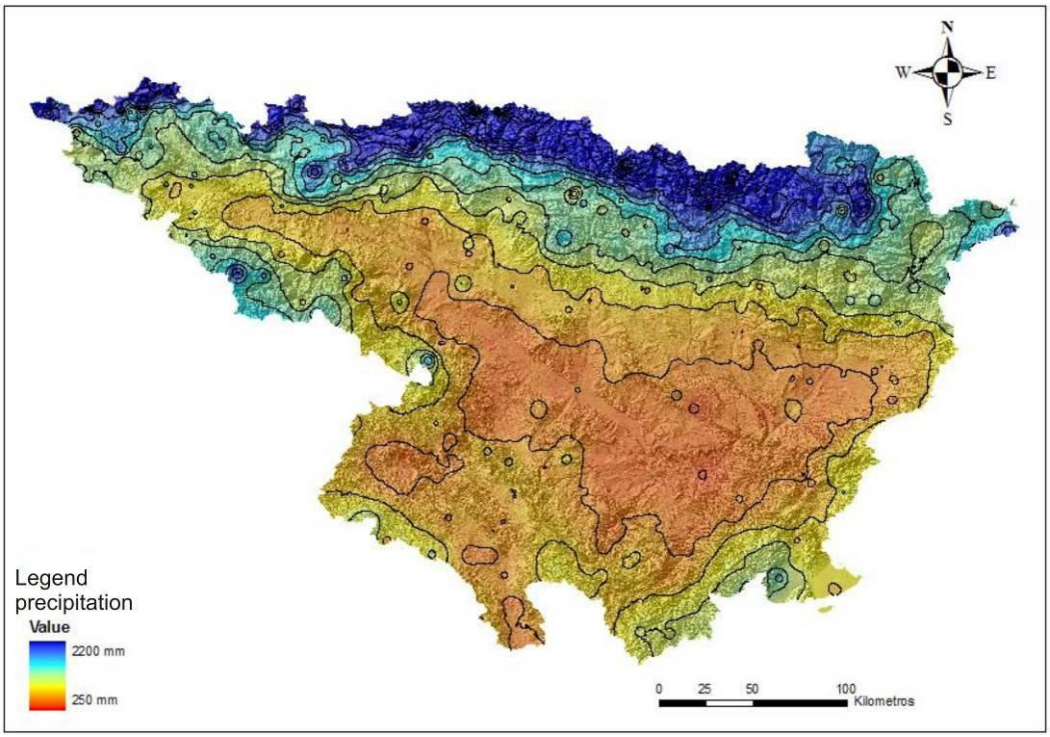


Figure 2. Mean annual precipitation in the Ebro basin (Extracted from CHE, 2005).

The context of the present thesis was carried out in the lower course of the Ebro River considering the last 100 km from river mouth, located in the Catalan part of the Ebro. This part of the river has a natural mean annual flow of 426 $m^3 \cdot s^{-1}$ at Ascó (Ebro Water Authority, URL: http//: www.chEbro.es) and is regulated by a complex of three large reservoirs built between 1940 and 1970: Mequinença, Riba-Roja and Flix, with a capacity of 1534 hm^3, 207 hm^3, and 11 hm^3, respectively (Figure 3). The presence of these reservoirs has changed the hydrology, geomorphology and ecology of the river by altering the magnitude, timing and duration of flows (Batalla et al., 2004), the sediment dynamics (Vericat & Batalla, 2006, 2007), the water temperature regimes and geochemistry (Ibáñez et al.,1995; Sabater et al.,2008; Prats et al., 2010). Therefore, river regulation is been considered the main anthropogenic impact in the system. A tendency of mean annual flow reduction of 29% has been reported during the 20th century, mainly attributed to irrigation usage but also to the evaporation occurring in the reservoirs (Ibáñez et al., 1996), which affects the salt wedge dynamics at the estuarine area. When

comparing recent hydrological data with the historical context, the actual situation is characterized by the reduction of high flows in autumn and winter, a slightly high flow in spring and early summer due to reservoir release after snowmelt in the Pyrenees, and by an increment of minimum flows in summer (Figure 4) (Sánchez & Ibáñez, 2008).

Figure 3. The lower Ebro River with the main reservoirs and delta (Extracted from Ibáñez et al., 1995).

During the last 20 years the dissolved nutrient concentrations presented a decreasing trend for phosphate, mostly attributed to the increase of waste water treatment plants in the basin, although the levels of nitrates were similar due to agriculture land use (Ibáñez et al., 2008). Consequently, together with the presence of zebra mussel (*Dreissena polymorpha* [Pallas]) in the reservoirs and the proliferation of macrophytes in the river section, a strong decrease in phytoplankton biomass has been reported (Sabater et al., 2008; Ibáñez et al., 2008).

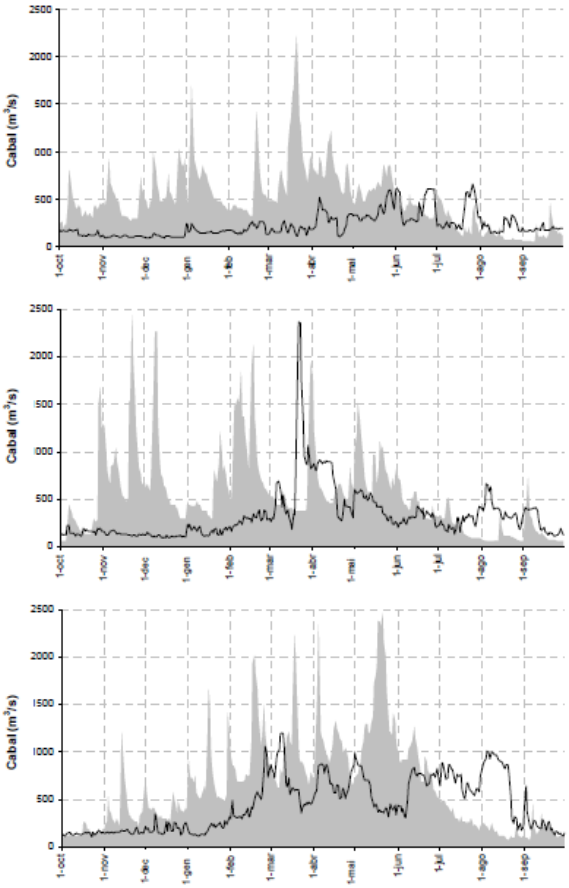


Figure 4. Water discharge in the lower Ebro River (Tortosa) under historical (grey) and actual conditions (black line) for dry hydrological years (upper figure), intermediate (central figure) and humid (lower figure) hydrological years.(Extracted from Sánchez & Ibáñez, 2008)

Within the other human impacts in the area, the presence of a nuclear power plant built in 1984 in Ascó, 5 km downstream of the lowermost dam, produces an increase of water temperature up to 3 ºC which can vary depending on the water discharge (Prats et al., 2010). Moreover, considering the effects of global warming (IPCC, 2007), an increase of 3.2 ºC has been predicted for 2070-2100 in the Ebro area (CHE, 2005), and effects on phenology of terrestrial species of birds, insects and plants have been already observed (Gordo & Sanz, 2005). Regarding pollution loading, the highest impact in the area is the deposition of more than 300,000 t of industrial solid wastes from alkali-chlorine electrolysis and phosphate fertilizer industry located in the lowermost reservoir at Flix, which mainly contains heavy metals and organochlorine compounds(Grimalt, 2006). The chemical plant in Flix was created in 1899 and was located nearby the Ebro River due to its strategic position in terms of raw material procurement and transport infrastructures (Figure 5; Sánchez & Visa, 1994). Due to the construction of the Flix reservoir in 1949, industrial waste products and natural sediments that previously were

transported by the river started to be deposited in the dam. However, since large floods were still possible due to the low capacity of the dam, only part of the material was retained. When the upstream Riba-Roja and Mequinença hydropower dams were built in the late 1960s, natural sediments were retained upstream of Flix and only industrial products were deposited. The largest quantity of polluted sediments was deposited from 1973 to 1984, due to the product from phosphate fertilizer, until the moment that waste of those products was banned. The pollutants from these sediments can be transported downstream (Gómez-Gutiérrez et al., 2006) and have been detected in organisms of the foodweb of the lower Ebro River, starting from the pollution source until the delta (Schuhmacher et al., 1993; Ramos et al 1999; Mañosa et al 2001; Sánchez-Chardi et al., 2007; Cid et al., 2010; Suarez-Serrano et al., 2010; Barata et al., 2010). To be brief, a general chronology of the main human impacts in the lower Ebro River since the beginning of the 20th century until today and their main effects in the ecosystem are presented in Figure 6.

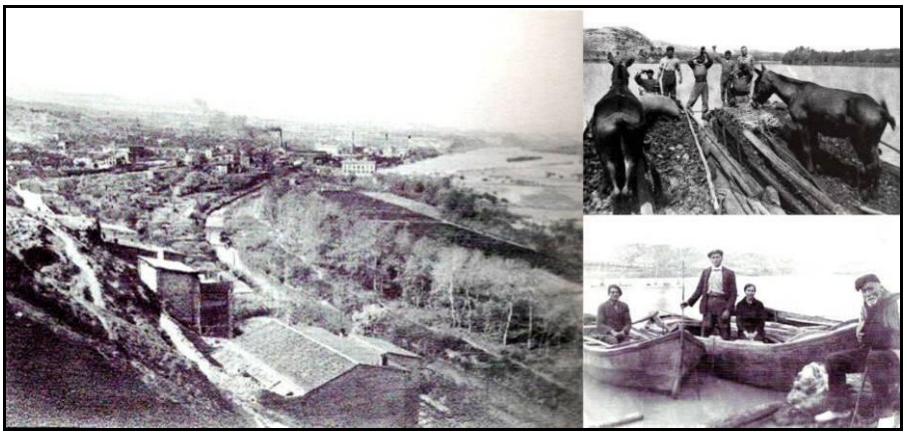


Figure 5. The chlor-alkali industry in Flix in the beginning of the 20th century (Extracted from Sanchez-Visa, 1994).

The study area in the present thesis was located within the freshwater part of the lower Ebro, extending from the Flix dam to the city of Tortosa. The lowermost areas which are under the influence of the salt wedge were not included since our study did not focus on communities from transitional waters (however, see Guillén et al., 1992; Ibáñez et al., 1997 for a detailed description of the estuary and its hydrological characteristics). At this part of the river, the riverbed is mainly composed of gravels, cobbles and sand in the interstices (Muñoz & Prat, 1994; Batalla & Vericat, 2009) and several fluvial islands

and side-arms can be present, providing higher habitat heterogeneity (Figure 7). However, the riparian vegetation covers a limited area at each margin of the river, since fluvial terraces have been modified by agriculture (see Limnos, 1998; Prats et al., 2010). Although the native vegetation is predominant, with species such as tamarisk (*Tamarix* sp.), poplar (*Populus nigra* Linnaeus), white poplar (*Populus alba* L.) and willows (*Salix* sp.), reed (*Phragmites australis* [Cav.] Steudel) and reedmace (*Typha* sp.), at places highly anthropized the main vegetation is composed by the introduced giant-reed (*Arundo donax* L.) (Fernández-González, 1990; Molina-Holgado, 2003; Curcó, 2007).

Since the beginning of the 80's, ecological and limnological research has been performed in the lower Ebro at the main reservoirs, the downstream freshwater part of the river, the estuary and delta, describing the main community structure and processes and assessing the main ecosystem impacts (de Sostoa et al., 1985; Muñoz, 1990; Muñoz & Prat, 1994; Ibáñez et al., 1995; Ibáñez et al., 1996; Limnos, 1998; Mañosa et al., 2001; Ibáñez & Prat, 2003; Val et al., 2003; Benejam et al., 2009; Cid et al., 2008, 2010; Suarez-Serrano et al., 2010; within others). Regarding the freshwater part of the lower Ebro, considered in the present thesis, previous descriptions of the community composition have been done, mainly on macroinvertebrates, ictiofauna, and phytoplankton. The macroinvertebrate community in this area was dominated by Chironomidae such as *Cricotopus* spp. and Oligochaeta, although filter-feeders such as *Hydropsyche exocellata* Duföur and *Ephoron virgo* [Olivier], collector-gatherers such as *Caenis* spp. and grazers such as *Theodoxus fluviatilis* L. were characteristic (Muñoz & Prat, 1994, Limnos, 1997). Nowadays, the macroinvertebrate community includes abundant populations of non-native species such as the zebra mussel (*D. polymorpha*) and the Asian clam (*Corbicula fluminea* [O.F. Müller]) (Jimenez-Muer, 2001; Sabater et al., 2008; Navarro et al., 2006), while native species such as the freshwater mussel *Margaritifera auricularia* (Spengler) are endangered and populations decline (Altaba, 1990; Araujo & Ramos, 2000). Populations of the blackfly *Simulim erythrocephalum* (De Geer) have become important in the river, mainly associated to the high proliferation of macrophyte beds they occupy, and causing problems of public health in the area. The fish community in the studied area is composed by a high proportion of alien species such as the European catfish (*Silurus glanis* L.) introduced in the late 60's into the reservoirs. The extintion of native anadromous species such as the Atlantic

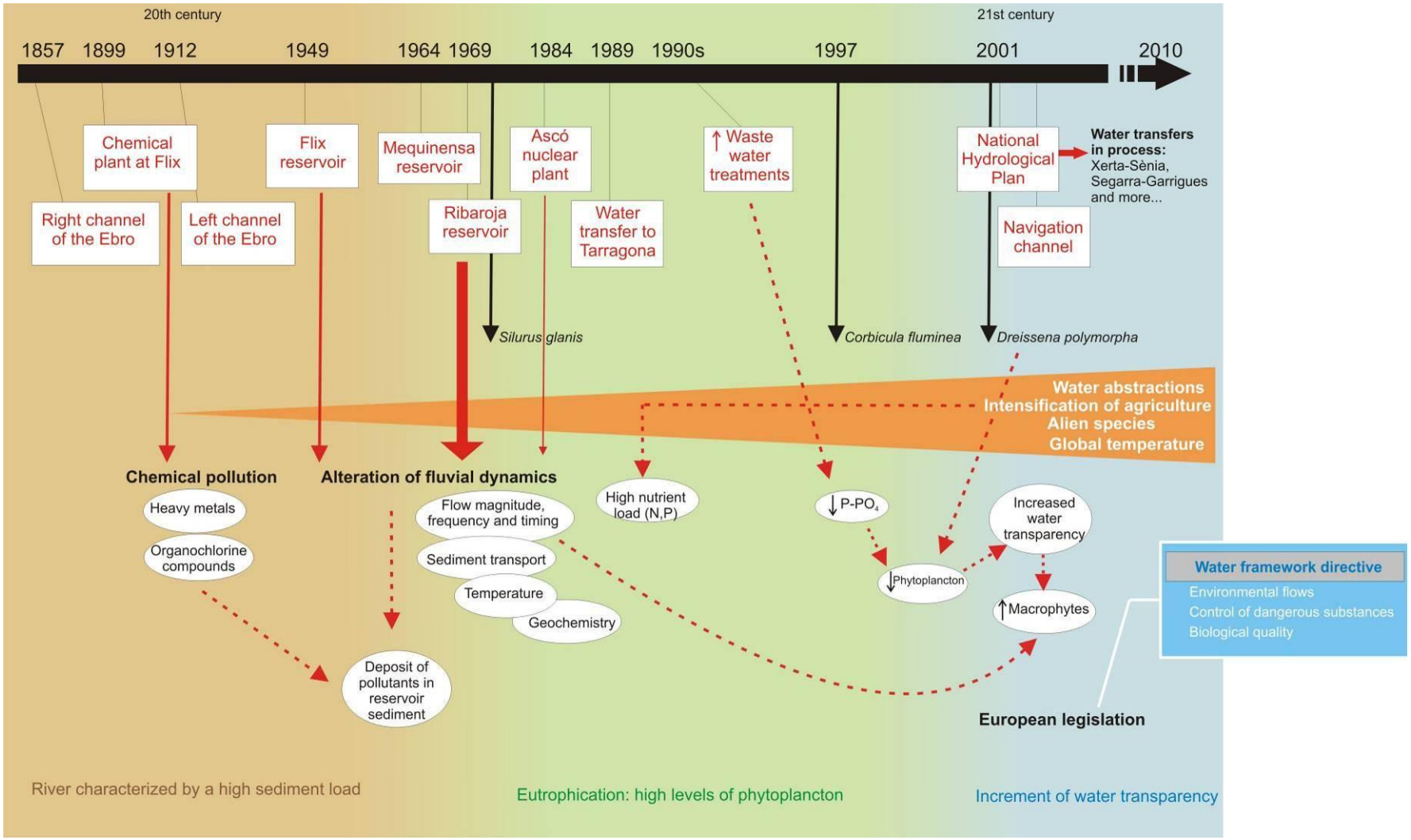


Figure 6. Chronology of the main human impacts in the lower Ebro River since the beginning of the 20th century until today and their main effects and interactions in the ecosystem.

Figure 7. The lower Ebro River: landscapes and substrates

sturgeon (*Acipenser sturio* L.), or the decline of twait shad (*Alosa fallax* [Lacepède]) has been mainly attributed to physical barriers, urban and industrial impacts and to river regulation (Sostoa & Lobon-Cervia, 1989; Fernández & Farnós, 1999), although recent data showed a slight population recovery of the twait shad in the area (López et al., 2007). Other important species are considered endemic (*Barbus graellsii* Steindachner and *Chondrostoma miegii* Steindachner), vulnerable (*Anguilla anguilla* L.) or threathened (*Salaria fluviatilis* Asso) (Doadrio, 2002; Ibáñez & Prat, 2003). Nowadays, the phytoplankton community of the Ebro below the reservoirs is very poor and mainly composed by centric diatoms and colonial and unicellular chlorophytes such as *Cyclotella* sp., *Stephanodiscus* sp., *Coelastrum microporum* Nägeli, *Pediastrum sp.*, *Gymnodinium* sp. and *Peridinium* sp.(Sabater & Muñoz, 1990; Sabater & Klee, 1990; Sabater et al., 2008). In the late 80s, although the reservoirs were already present, the high dissolved phosphate concentrations and the absence of zebra mussel in the reservoirs propitiated a more abundant community although their actual composition appears to be similar. On the other hand, the benthic algal community is mainly

dominated by *Cladophora* sp. and by a diverse diatom community with species such as *Amphora pediculus* (Kützing) Grunow, *Cocconeis placentula* var. *lineata* Ehrenbergh, *Navicula cryptotenella* Lange-Bertalot, *Nitzschia dissipata* (Kützing) Grunow, *N. palea* (Kützing) W.Smith and *N. incosnpicua* Grunow among the most abundant ones (Rosa Trobajo personal communication). Average benthic chlorophyll *a* algal concentrations were recorded as 50-250 mg/m^2 in 2005 and 2006 (Sabater et al., 2008). The proliferation of macrophyte beds throughout the lower part of river during the last 10 years is also of important concern since induces ecological changes that affect public health and economy in the area (Ibañez et al., 2008; Batalla et al., 2009). The reported macrophyte taxa are *Potamogeton pectinatus* L., *Myriophyllum spicatum* L., *Ceratophyllum demersum* L. (Limnos, 1998; Andreu, 2007), mainly present in slow-flow areas although *P. pectinatus* may be found in all habitats.

With all this in mind, the different type of impacts and pressures suffered by the lower Ebro freshwater aquatic ecosystem are integrated in the present thesis considering the effect on aquatic macroinvertebrates at different levels of organization. In the following section, the main objectives and structure of the thesis are presented.

References

Adams, S.M. & Greeley, M.S. (2000) Ecotoxicological indicators of water quality: Using multi-response indicators to assess the health of aquatic ecosystems. *Water Air and Soil Pollution,* **123,** 103-115.

Allan, J.D. & Castillo, M.M. (2008) *Stream ecology, structure and function of running waters,* Springer, Dordrecht, The Netherlands.

Altaba, C.R. (1990) The last known population of the freshwater mussel *Margaritifera auricularia* (Bivalvia, Unionida): a conservation priority. *Biological Conservation,* **52,** 271-286.

Andreu, R. (2007) Colonització per macròfits i els seus macroinvertebrats associats al tram baix del riu Ebre. Master thesis. Department of Ecology, University of Barcelona. p 57.

Araujo, R. & Ramos, M.A. (2000) Status and conservation of the giant European freshwater pearl mussel (*Margaritifera auricularia*) (Spengler, 1793) (Bivalvia : Unionoidea). *Biological Conservation,* **96,** 233-239.

Barata, C., Fabregat, M.C., Cotin, J., Huertas, D., Sole, M., Quiros, L., Sanpera, C., Jover, L., Ruiz, X., Grimalt, J.O. & Piña, B. (2010) Blood biomarkers and contaminant levels in feathers and eggs to assess environmental hazards in heron nestlings from impacted sites in Ebro basin (NE Spain). *Environmental Pollution,* **158,** 704-710.

Barata, C., Lekumberri, I., Vila-Escalé, M., Prat, N. & Porte, C. (2005) Trace metal concentration, antioxidant enzyme activities and susceptibility to oxidative stress in the tricoptera larvae *Hydropsyche exocellata* from the Llobregat River basin (NE Spain). *Aquatic Toxicology,* **74,** 3-19.

Batalla, R.J., Gómez, C.M. & Kondolf, G.M. (2004) Reservoir-induced hydrological changes in the Ebro River basin (NE Spain). *Journal of Hydrology,* **290,** 117-136.

Batalla, R.J. & Vericat, D. (2009) Hydrological and sediment transport dynamics of flushing flows: implications for management in large Mediterranean rivers. *River Research and Applications,* **25,** 297-314.

Benejam, L., Benito, J. & García-Berthou, E. (2010) Decreases in condition and fecundity of freshwater fishes in a highly polluted reservoir. *Water Air and Soil Pollution,* **210,** 231-242.

Bonada, N., Prat, N., Resh, V.H. & Statzner, B. (2006) Developments in aquatic insect biomonitoring: A comparative analysis of recent approaches. *Annual Review of Entomology,* **51,** 495-523.

Bonada, N., Vives, S., Rieradevall, M. & Prat, N. (2005) Relationship between pollution and fluctuating asymmetry in the pollution-tolerant caddisfly *Hydropsyche exocellata* (Trichoptera, Insecta). *Archiv Fur Hydrobiologie,* **162,** 167-185.

Bonada, N., Zamora-Muñoz, C., Rieradevall, M. & Prat, N. (2004) Ecological profiles of caddisfly larvae in Mediterranean streams: implications for bioassessment methods. *Environmental Pollution,* **132,** 509-521.

Buchwalter, D.B., Cain, D.J., Clements, W.H. & Lucima, S.N. (2007) Using biodynammic models to reconcile differences between laboratory toxicity tests and field biomonitoring with aquatic insects. *Environmental Science & Technology,* **41,** 4821-4828.

Cain, D.J., Buchwalter, D.B. & Luoma, S.N. (2006) Influence of metal exposure history on the bioaccumulation and subcellular distribution of aqueous cadmium in the insect *Hydropsyche californica*. *Environmental Toxicology and Chemistry,* **25,** 1042-1049.

Cain, D.J., Luoma, S.N. & Wallace, W.G. (2004) Linking metal bioaccumulation of aquatic insects to their distribution patterns in a mining-impacted river. *Environmental Toxicology and Chemistry,* **23,** 1463-1473.

Cid, N., Ibáñez, C., Palanques, A. & Prat, N. (2010) Patterns of metal bioaccumulation in two filter-feeding macroinvertebrates: exposure distribution, inter-species differences and variability across developmental stages. *Science of the Total Environment,* **408,** 2795-2806.

CHE (2005) Evaluación de los impactos del cambio climático en los recursos hídricos del río Ebro. Available at http://www.chebro.es/contenido.visualizar.do?idContenido=14281&idMenu=3084

Curcó, A. (2007) La flora vascular del Delta de l'Ebre. Generalitat de Catalunya, Departament de Medi Ambient I Habitatge. Available at http://mediambient.gencat.net/Images/43_155961.pdf

De Lange, H.J., De Haas, E.M., Maas, H. & Peeters, E. (2005) Contaminated sediments and bioassay responses of three macroinvertebrates, the midge larva *Chironomus riparius*, the water louse *Asellus aquaticus* and the mayfly nymph *Ephoron virgo*. *Chemosphere,* **61,** 1700-1709.

De Sostoa, A., De Sostoa, F.J. & Yanez-Arancibia, A. (1985) The fish communities of the Ebro Delta (northeast Spain): a model of Mediterranean estuarine ecosystem. *Fish community ecology in estuaries and coastal lagoons.*, 79-126.

De Sostoa, A. & Lobón-Cervia, J. (1989) Fish and fisheries of the river Ebro (Spain), actual state and recent history. *Petts, G. E., H. Moeller and A. L. Roux (Ed.).* Historical Change of Large Alluvial Rivers: Western Europe. 355p. John Wiley and Sons, Inc.: Somerset, New Jersey, USA; Chichester, England, Uk.

Diggins, T.P. & Stewart, K.M. (1998) Chironomid deformities, benthic community composition, and trace elements in the Buffalo River (New York) Area of Concern. *Journal of the North American Benthological Society,* **17,** 311-323.

Doadrio, I. (2002) Atlas y libro rojo de los peces continentales de España. Ministerio de Medio Ambiente, Madrid, p 374.

Dolédec, S., Statzner, B. & Bournard, M. (1999) Species traits for future biomonitoring across ecoregions: patterns along a human-impacted river. *Freshwater Biology,* **42,** 737-758.

European Commission. 2000. Directive 2000/60/EC of the European Parliament and of the Council. Establishing a framework for Community action in the field of water policy, Brussels, Belgium.

Fernandez-González, F., Molina, A. & Loidi, J. (1990) Los tarayales de la depressión del Ebro. Acta Botanica Malacitana, 15, 311-322.

Gómez-Gutiérrez, A.I., Jover, E., Bodineau, L., Albaiges, J. & Bayona, J.M. (2006) Organic contaminant loads into the Western Mediterranean Sea: Estimate of Ebro River inputs. *Chemosphere,* **65,** 224-236.

Gordo, O. & Sanz, J.J. (2005) Phenology and climate change: a long-term study in a Mediterranean locality. *Oecologia,* **146,** 484-495.

Grimalt, J.O., 2006. Available at http://mediambient.gencat.cat/cat/ciutadans/informacio_ambiental/Flix/estudi.jsp?ComponentID=42291&SourcePageID=42851#1

Guillén, J. & Palanques, A. (1992) Sediment dynamics and hydrodynamics in the lower course of a river highly regulated by dams: the Ebro River. *Sedimentology,* **39,** 567-579.

Hare, L., Tessier, A. & Warren, L. (2001) Cadmium accumulation by invertebrates living at the sediment-water interface. *Environmental Toxicology and Chemistry,* **20,** 880-889.

Henle, K., Scholz, M., Dziock, F., Stab, S. & Foeckler, F. (2006) Bioindication and functional response in floodplain systems: Where to from here? *International Review of Hydrobiology,* **91,** 380-387.

Ibáñez, C., Pont, D. & Prat, N. (1997) Characterization of the Ebre and Rhone estuaries: a basis for defining and classifying salt-wedge estuaries. *Limnology and Oceanography,* **42,** 89-101.

Ibáñez, C. & Prat, N. (2003) The environmental impact of the Spanish National Hydrological Plan on the lower Ebro River and delta. *International Journal of Water Resources Development,* **19,** 485-500.

Ibáñez, C., Prat, N. & Canicio, A. (1996) Changes in the hydrology and sediment transport produced by large dams on the lower Ebro River and its estuary. *Regulated Rivers-Research & Management,* **12,** 51-62.

Ibáñez, C., Prat, N., Duran, C., Pardos, M., Munné, A., Andreu, R., Caiola, N., Cid, N., Hampel, H., Sanchez, R. & Trobajo, R. (2008) Changes in dissolved nutrients in the lower Ebro River: Causes and consequences. *Limnetica,* **27,** 131-142.

Ibáñez, C., Rodrigues-Capitulo, A. & Prat, N. (1995) The combined impacts of river regulation and eutrophication on the dynamics of the salt wedge and the ecology of the lower Ebro River (North-East Spain). *Ecological Basis for River Management***,** 105-113.

IPCC (2007) Climate change 2007: impacts, adaptation and vulnerability. Summary for policymakers, p 22.

Jiménez Muer, P. (2001) Localization and evaluation of a new biological invasion: the zebramussel (*Dreissena polymorpha*) in the Ebro. Available at www.agua-debate.org/htm/mcebra/docmc/mma_freixe.pdf2001.

Klok, C. & Kraak, M.H.S. (2008) Living in highly dynamic polluted river floodplains, do contaminants contribute to population and community effects? *Science of the Total Environment,* **406,** 455-461.

Lacorte, S., Raldúa, D., Martinez, E., Navarro, A., Díez, S., Bayona, J.M. & Barceló, D. (2006) Pilot survey of a broad range of priority pollutants in sediment and fish from the Ebro river basin (NE Spain). *Environmental Pollution,* **140,** 471-482.

Limnos, 1997. Estudi dels efectes de l'abocament tèrmic de la central nuclear d'Ascó sobre les comunitats biològiques. Junta de Sanejament, Barcelona. 50 p.

Limnos, 1998. Estudio de la calidad ecológica integral del río Ebro. http://oph.chebro.es/DOCUMENTACION/Calidad/estudios/calecolebro1998/indexh.htm

López, M.A., Gazquez, N., Olmo-Vidal, J.M., Aprahamian, M.W. & Gisbert, E. (2007) The presence of anadromous twaite shad (*Alosa fallax*) in the Ebro River (western Mediterranean, Spain): an indicator of the population's recovery? *Journal of Applied Ichthyology,* **23,** 163-166.

Mañosa, S., Mateo, R. & Guitart, R. (2001) A review of the effects of agricultural and industrial contamination on the Ebro delta biota and wildlife. *Environmental Monitoring and Assessment,* **71,** 187-205.

Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-being: Biodiversity Synthesis. World Resources Institute, Washington, DC. Available at http://www.millenniumassessment.org/en/Synthesis.aspx

Molina- Holgado, P. (2003) Análisis y comparación de la vegetación de las riberas de los ríos Ebro, Tajo y Jarama. UAM Ediciones-Obra Social Caja Madrid. Madrid.

Muñoz, I. (1990) Limnologia de la part baixa del riu Ebre i els canals de reg: els factors fisicoquímics, el fitoplancton i els macroinvertebrats bentònics. PhD thesis. University of Barcelona.

Muñoz, I. & Prat, N. (1994) Macroinvertebrate community in the lower Ebro River (NE Spain) *Hydrobiologia,* **286,** 65-78.

Navarro, E., Bacardit, M., Caputo, L., Palau, T. & Armengol, J. (2006) Limnological characterization and flow patterns of a three-coupled reservoir system and their influence on *Dreissena polymorpha* populations and settlement during the stratification period. *Lake and Reservoir Management,* **22,** 293-302.

Petts G.E., H. Möller & A.L. Roux (Eds). 1993. Historical change of alluvial rivers: Western Europe. John Wiley & Sons, New York. 355p.

Poff N.L., J.D. Olden, D.M. Merritt & Pepin, D.M. (2007) Homogenization of regional river dynamics by dams and global biodiversity implications. *Proceedings of the National Academy of Sciences of the United States of America,* **104**, 5732–5737.

Prats, J., Val, R., Armengol, J. & Dolz, J. Temporal variability in the thermal regime of the lower Ebro River (Spain) and alteration due to anthropogenic factors. *Journal of Hydrology,* **387,** 105-118.

Pujol, L. & Sánchez-Cabeza, J.A. (2000) Natural and artificial radioactivity in surface waters of the Ebro river basin (Northeast Spain). *Journal of Environmental Radioactivity,* **51,** 181-210.

Ramos, L., Fernández, M.A., González, M.J. & Hernández, L.M. (1999) Heavy Metal Pollution in Water, Sediments, and Earthworms from the Ebro River, Spain. *Bulletin of Environmental Contamination and Toxicology [Bull. Environ. Contam. Toxicol.]. Vol. 63***,** no. 3.

Rosenberg, D. M. & V. H. Resh. 1993. Freshwater biomonitoring and benthic macroinvertebrates. Chapmann and Hall, New York. 488p.

Sabater, S., Artigas, J., Durán, C., Pardos, M., Romaní, A.M., Tornes, E. & Ylla, I. (2008) Longitudinal development of chlorophyll and phytoplankton assemblages in a regulated large river (the Ebro River). *Science of the Total Environment,* **404,** 196-206.

Sabater, S. & Klee, R. (1990) Observations on centric diatoms on the River Ebro (Spain): phytoplankton with special interest on some small *Cyclotella*. *Diatom Research,* **5,** 141-154.

Sabater, S. & Muñoz, I. (1990) Successional dynamics of the phytoplankton in the lower part of the River Ebro. *Journal of Plankton Research,* **12,** 573-592.

Sánchez, R. & Ibáñez, C. Agència Catalana de l'Aigua. Available at http://acaweb.gencat.cat/aca/documents/ca/planificacio/cabals/cabals_ambientals_baix_ebre_2008.pdf.

Sánchez - Cervelló, J. & Visa - Ribera, F.R. (1994) *La navegació fluvial i la industrialització a Flix. 1840-1940.,* La Veu de Flix. Flix.

Sánchez - Chardi, A., López- Fuster, M.J. & Nadal, J. (2007) Bioaccumulation of lead, mercury, and cadmium in the greater white-toothed shrew, *Crocidura russula*, form the Ebro Delta (NE Spain): Sex- and age- dependent variation. *Environmental Pollution,* **145,** 7-14.

Schuhmacher, M., Domingo, J.L., Llobet, J.M. & Corbella, J. (1993) Evaluation of the effect of temperature, pH, and bioproduction on Hg concentration in sediments, water, molluscs and algae of the delta of the Ebro river. *Science of the Total Environment,* **0,** 117-125.

Skinner, K.M. & Bennett, J.D. (2007) Altered gill morphology in benthic macroinvertebrates from mercury enriched streams in the Neversink Reservoir Watershed, New York. *Ecotoxicology,* **16,** 311-316.

Statzner, B., Bady, P., Dolédec, S. & Scholl, F. (2005) Invertebrate traits for the biomonitoring of large European rivers: an initial assessment of trait patterns in least impacted river reaches. *Freshwater Biology,* **50,** 2136-2161.

Statzner, B. & Beche, L.A. (2010) Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? *Freshwater Biology,* **55,** 80-119.

Strayer, D.L., Pace, M.L., Caraco, N.F., Cole, J.J. & Findlay, S.E.G. (2008) Hydrology and grazing jointly control a large-river food web. *Ecology,* **89,** 12-18.

Suarez-Serrano, A., Alcaraz, C., Ibáñez, C., Trobajo, R. & Barata, C. (2010) *Procambarus clarkii* as a bioindicator of heavy metal pollution sources in the lower Ebro River and Delta. *Ecotoxicology and Environmental Safety,* **73,** 280-286.

Terrado, M., Barceló, D. & Tauler, R. (2006) Identification and distribution of contamination sources in the Ebro basin by chemometrics modelling coupled to geographical information systems. *Talanta,* **70,** 691-704.

Tockner, K., Robinson, C.T. & Uehlinger, U. (2009) Rivers of Europe, Academic press, London. 728p.

Townsend, C.R. & Hildrew, A.G. (1994) Species traits in relation to a habitat templet for river systems *Freshwater Biology,* **31,** 265-275.

Val, R., Niñerola, D., Armengol, J. & Dolz, J. (2003) Incidencia de los embalses en el régimen térmico del río. El caso del tramo final del río Ebro. *Limnetica,* **22,** 85-92.

Vericat, D. & Batalla, R.J. (2006) Sediment transport in a large impounded river: The lower Ebro, NE Iberian Peninsula. *Geomorphology,* **79,** 72-92.

Vericat, D. & Batalla, R.J. (2007) Fractional bedload transport during small floods in a regulated gravel-bed river. *Zeitschrift Fur Geomorphologie,* **51,** 227-240.

Objectives

The general objective of the present study was to assess the effects of the main environmental stressors on the freshwater aquatic ecosystem of the lower Ebro River using the benthic macroinvertebrate community as bioindicator. To achieve this objective, different organization levels (i.e. community, population and individual) and different approaches were integrated (Figure 8) in function of the type of stressor.

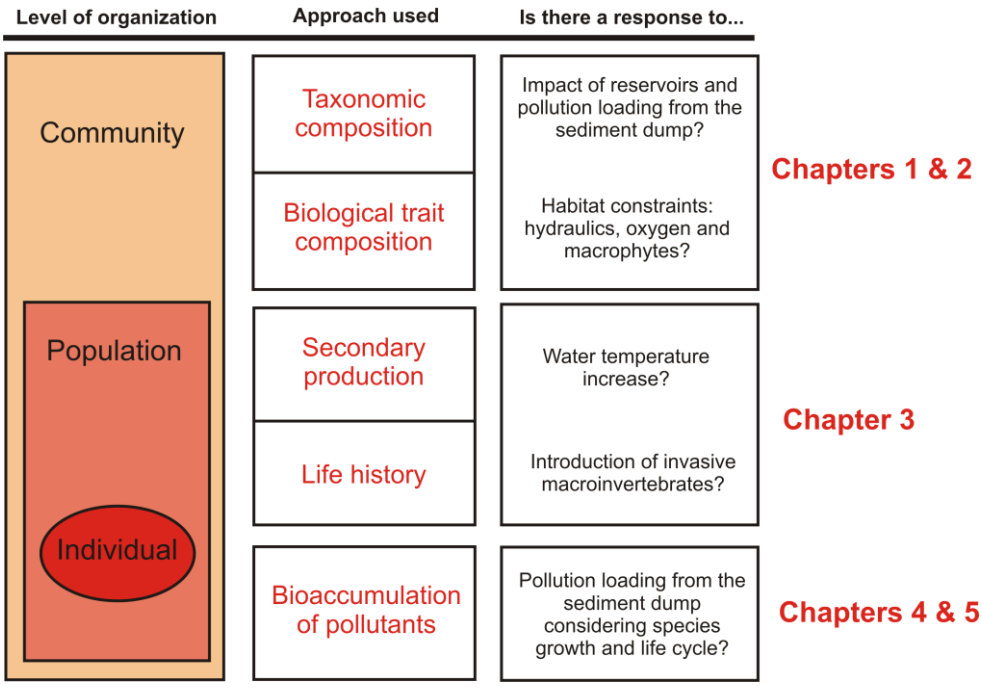


Figure 8. Organization of the chapters according to level of organization, approach used and main questions of the study.

The thesis was divided into 5 chapters, corresponding to 5 article manuscripts (two of them already published, one submitted, and two to be submitted). More concretely, each chapter aimed to answer the following questions:

Chapter 1. Is the taxonomic and biological trait composition of the community influenced by the distance from the reservoirs and dump of polluted sediments? Is there a temporal variability? Are community metrics influenced by these factors?

Chapter 2. Do habitat constraints such as hydraulics, oxygen and macrophyte cover determine the macroinvertebrate assemblages? Which are the hydraulic preferences of the community? Is taxonomy resolution important to asses these preferences? Is there a biological trait response to these environmental factors? Are community metrics influenced by hydraulics?

Chapter 3. Is there an influence of water temperature change on the secondary production and life history of aquatic insects? Is there a change in populations of native filter-feeders such as *Ephoron virgo* after the establishment of alien species with same feeding habits (*Corbicula fluminea* and *Dreissena polymorpha*)?

Chapter 4 & 5. Is there a bioaccumulation of pollutants in the freshwater ecosystem of the Ebro River? Are these pollutants originated from the Flix sediment dump? Which are the patterns of bioaccumulation along growth and life cycle stages of sensitive species as *Ephoron virgo*? Are there differences between the compounds analyzed? Are there differences in bioaccumulation between sensitive (*Ephoron virgo*) and tolerant (*Hydropsyche exocellata*) species?

The answers to these questions were developed at each chapter and integrated in the overall discussion. Finally, the concluding remarks and future perspectives were presented.

Resum de les publicacions (*in Catalan*)

Article 1. Estudi comparatiu de l'anàlisi de la taxonomia i dels trets biològics dels macroinvertebrats: importància de la regulació del cabal i la contaminació de sediments al tram baix del riu Ebre

Paraules clau: Macroinvertebrats, trets biològics, riu Ebre, grans rius, diversitat funcional

Els cursos dels grans rius estan sotmesos a l'impacte de múltiples pressions antropogèniques. En aquest estudi, les condicions hidrològiques i la contaminació de metalls pesants i compostos organoclorats a l'embassament de Flix van ser considerats per explicar els canvis temporals i espaials de la comunitat de macroinvertebrats bentònics i els seus trets biològics. Les diferents situacions hidrològiques a la primavera i la tardor van explicar una major variabilitat dels trets biològics en comparació amb la composició taxonòmica, que només variava lleugerament d'acord a les escales temporals i espacials. Si bé després d'un esdeveniment de cabals relativament elevats (primavera) la comunitat es caracteritzava per una major presència de trets típics de resistència / resiliència (alta taxa de reproducció, augment de la mobilitat), després d'un període de cabals relativament baixos i constants la composició funcional era més diversa degut a un major nombre de trets biològics. En conseqüència, la riquesa i diversitat funcional de la comunitat va ser més elevada sota condicions estables. Malgrat l'elevada variabilitat temporal dels trets biològics, la composició de certs trets biològics era diferent seguint un gradient de disminució dels impactes aigües avall de les preses i la contaminació de Flix. Les proporcions de filtradors que excaven el sediment i s'alimenten de detritus fi augmentaven com més elevada era la distància dels impactes, mentre que els depredadors i aquells que es

reprodueixen asexualment seguien un patró oposat. Al llarg d'aquest gradient, les mètriques funcionals basades amb els trets biològics no van mostrar una resposta evident. Malgrat això, els valors de les mètriques estructurals basades amb la taxonomia dels macroinvertebrats, incloent els índexs biològics com l'IBMWP, incrementaven a mesura que la distància des de la presa de Flix augmentava, a causa de la presència d'espècies relativament sensibles com l'*Ephoron virgo* o *Choroterpes pictetii*. En general, els nostres resultats van mostrar que les diferents condicions hidrològiques van condicionar els altres impactes com la contaminació (per exemple, metalls pesants i organoclorats contaminació) ja que l'efecte combinat d'un cabal elevat pot causar una exposició més elevada als contaminants degut a una major remobilització de sediments des de l'embassament.

Article 2. *Les condicions hidràuliques com a element clau per a les comunitats de macroinvertebrats bentònics, la composició dels trets biològics i la diversitat en un riu regulat.*

Paraules clau: preferències hidràuliques, trets biològics, diversitat, macroinvertebrats, Chironomidae, riu Ebre

Atès que les condicions hidràuliques són un dels principals factors que determinen la distribució dels macroinvertebrats aquàtics en rius, les preferències de cada espècie i la seva estructura funcional basada amb els seus trets biològics es van caracteritzar al llarg d'un gradient hidràulic al tram baix del riu Ebre mitjançant l'anàlisi OMI (de l'anglès, Outlier Mean Index o també anomenat Niche analysis) i el mètode d'anàlisi quarta cantonada (de l'anglès, Fourth Corner Method). Al riu, es van agafar mostres quantitatives de macroinvertebrats bentònics al mateix temps que la velocitat mitjana de l'aigua, la profunditat, el percentatge de cobertura dels macròfits i l'oxigen dissolt al bentos. El nombre de Froude i el nombre de Reynolds també es van calcular. De tots els paràmetres mesurats, la velocitat mitjana d'aigua va ser la millor variable explicativa de la distribució de macroinvertebrats i de l'estructura funcional. Totes les mètriques, tant estructurals com funcionals, es van correlacionar negativament amb la velocitat del corrent, mentre que la densitat de macroinvertebrats va mostrar una tendència oposada, a causa de la presència d'unes quantes espècies dominants en àrees on la velocitat de l'aigua era elevada. Els tàxons que presentaren una elevada marginalitat pel que fa al

seu nínxol ecològic (en aquest cas, basat en la hidràulica) mostraven una disponibilitat d'hàbitat més estreta (àrees amb ràpids i lents) mentre que altres espècies presentaven una tolerància més elevada a un ampli rang de condicions. Concretament, el grup dels Chironomidae van mostrar diferents preferències hidràuliques dins de la mateixa subfamília, tribu o gènere, reflectint la importància específica per la delimitació del nínxol ecològic. D'acord amb la distribució de macroinvertebrats, moltes de les característiques funcionals van respondre a les condicions hidràuliques (per exemple, alimentació, locomoció), mentre que les relacionades amb el cicle vital podrien reflectir més aviat adaptacions al règim hidrològic o interaccions entre espècies. Al vincular la diversitat i riquesa de la comunitat de macroinvertebrats amb les seves preferències hidràuliques i la resposta dels seus trets biològics, es va demostrar que la realització d'aquest tipus d'estudis amb una elevada resolució taxonòmica millorarà la nostra comprensió de la resposta de la comunitat a les condicions hidràuliques i per tant augmentarà el seu potencial per a la seva aplicació la gestió del cabal ecològic dels rius.

Article 3. Cicle vital i producció de la palometa *Ephoron virgo* (Olivier, 1791) (Ephemeroptera: Polymitarcyidae) al tram baix del riu Ebre: comparativa després de 18 anys.

Paraules clau: *Ephoron virgo*; cicle vital; producció secundària; temperatura; riu Ebre

El cicle biològic de l'*Ephoron virgo* (Ephemeroptera: Polymitarcyidae) es va estudiar durant la primavera i l'estiu de 2005 al tram baix del riu Ebre en comparació amb un estudi previ realitzat en 1987 (Ibáñez et al., 1991). Els resultats van mostrar un avanç del cicle biològic i un augment de les estimacions de producció. Al 2005, el desenvolupament de les nimfes va arribar a una mida màxima un mes abans que al 1987, i el pic d'emergències d'adults es va iniciar tres setmanes abans. La comparació de la relació entre femelles i mascles adults (F: M), va resultar en una important predominància de femelles al 2005 (1:4), mentre que al 1987 es va observar el contrari (2:1). La producció secundària d'aquesta espècie va ser més elevada al 2005 que al 1987, obtenint 950 mg pes sec/m^2/any utilitzant el mètode de l'increment sumatori (de l'anglès, Increment Summation Method) i 1080 mg pes sec/m^2/any, utilitzant el mètode de l'extracció sumatòria (de l'anglès, Removal Summation Method). Durant l'etapa del creixement de les nimfes al llarg de 2005 es van detectar temperatures de l'aigua més

elevades que al 1987, en correlació amb una temperatura de l'aire també més elevada. Per tant, l'increment de la temperatura va ser en major part la causa principal dels canvis observats en el cicle de vida *Ephoron virgo*.

Article 4. Pautes de bioacumulació de metalls en dos macroinvertebrats filtradors: distribució espaial, diferències interespecífiques i variabilitat al llarg del desenvolupament

Paraules clau: metalls pesats; *Ephoron virgo*; Hydropsyche; cicle vital; riu Ebre

Aquest estudi es va basar en la bioacumulació de metalls de dos insectes aquàtics (*Ephoron virgo* i *Hydropsyche* spp.) per tal d'avaluar la distribució espacial dels metalls, les diferències interespecífiques entre ambdós filtradors i la dinàmica de bioacumulació en les etapes de desenvolupament d'*E. virgo*. Els metalls Hg, Cd, Ni, Cr, As, Pb, Cu, Ti, Zn i Mn es van quantificar en els insectes i en les partícules en suspensió, ambdós mostrejats aigües avall i aigües amunt d'una planta química a la localitat de Flix, on hi són dipositats més de 300.000 tones de sediments contaminats. Les concentracions de mercuri van ser un ordre de magnitud superior aigües avall dels sediments contaminats, demostrant que la contaminació de Hg té el seu origen a la planta química. En canvi, el Cd, Ni, Cr, Pb, Ti, Zn i Mn en els invertebrats aquàtics mesurats va mostrar que contaminació per metalls ja era present aigües amunt en altres parts del riu, malgrat no tenir concentracions tan elevades. Es van observar diferències interespecífiques en la bioacumulació de tots els metalls analitzats, excepte per al Mn. Les concentracions en l'*E. virgo* van ser significativament més altes que en l' *Hydropsyche exocellata*, destacant el Cd, que va mostrar valors deu vegades més grans. Quan es va analitzar el patró de bioacumulació dels metalls al llarg del cicle de l'*E. virgo*, l'Hg i el Cd van augmentar paulatinament fins que les nimfes van assolir 11 mm i posteriorment en els últims estadis quan les nimfes estadis estaven a punt d'emergir. En canvi, el Cr, Pb, Ti i Mn van disminuir al llarg dels primers estadis de creixement, seguit d'un estat d'equilibri fins els estadis finals. Per al Cu, As i Zn es va obtenir valors similars al llarg de tots el creixement. Les diferències de bioacumulació entre els mascles i les femelles adults d'*E. virgo* van ser marcades per al Cd, Cu i Mn. La persistència de l'Hg i del Cd va ser més elevada al llarg dels diferents estadis de desenvolupament ja que es van detectar concentracions relativament elevades en els ous i en els adults. Com que el

comportament dels tots els metalls analitzats va ser diferent en les dues espècies i durant les etapes del cicle biològic de l'*E. virgo*, aquests resultats haurien de ser considerats en la interpretació de la concentració de metalls en insectes aquàtics quan s'avalua el risc de transferència dels metalls al llarg de la cadena alimentària dels ecosistemes fluvials.

Article 5. Bioacumulació de compostos organoclorats en l'efemeròpter filtrador *Ephoron virgo* al llarg del seu cicle biològic en un riu amb contaminació crònica.

Paraules clau: compostos organoclorats; *Ephoron virgo*; cicle biològic; riu Ebre

El total dels compostos organoclorats (OC), penta-i HEXA-clorobenzè, (PECB i HCB) hexaclorociclohexans (HCH), dicloro-tricloroetans (DDT) i policlorobipenyls (PCB), es van avaluar al llarg de les etapes del cicle biològic de l'efemeròpter *Ephoron virgo* en el riu Ebre, on l'ecosistema rep una contaminació crònica per aquests compostos a causa de la presència dels sediments tòxics a l'embassament de Flix. Aquest organisme va ser seleccionat per la seva alta sensibilitat als contaminants, la seva rellevància ecològica, i el seu potencial de bioacumulació com a filtrador. La bioacumulació dels contaminants es va avaluar mitjançant el contrast dels nivells d'organoclorats aigües avall del focus de contaminació comparant-los am els d'un punt de control aigües amunt. L'augment dels valors de HCB, HCH, DDE i PCB al llarg del creixement de les nimfes van demostrar la bioconcentració d'aquests compostos durant el creixement. Els adults d'aquesta espècie capturats durant les emergències van mostrar els nivells més alts d'organoclorats, amb augments de 2 a 8 vegades el valor que tenien les nimfes. Aquest fet pot tenir implicacions importants en avaluar la transferència d'aquests contaminants a nivells tròfics superiors, ja els adults són una font d'aliment més accessible per a molts tipus de depredadors. Les diferències de bioacumulació entre els mascles i les femelles adults d'*E. virgo* van ser marcades per al DDE i PCB, on els mascles tenien concentracions més elevades. No obstant això, i com a conseqüència d'una baixa transmissió materna de contaminants, els ous contenien unes concentracions fins a 5 vegades menys que el valor de les mares. Els resultats van demostrar una important variabilitat en la bioacumulació de compostos organoclorats al llarg del cicle biològic d'aquesta espècie, i per tant, és un factor important a tenir en compte quan s'avalua el risc de transferència de contaminants en ecosistemes aquàtics i riberencs, i quan es vol establir un vincle entre els resultats procedents d'estudis ecotoxicològic en condicions

de laboratori amb les de les condicions en l'hàbitat natural. En general, l'elevada concentració de compostos organoclorats detectats en l'espècie estudiada posa de manifest el risc ecològic de les comunitats aquàtiques en la part baixa del riu Ebre.

Chapter 1

Comparative use of taxonomy and trait-based approaches in macroinvertebrates: importance of flow regulation and sediment pollution in the lower Ebro River

Cid, N., Ibánez, C., Andreu, R. & Prat, N.

***Hydrobiologia* (to be submitted).**

Comparative use of taxonomy and trait-based approaches in macroinvertebrates: importance of flow regulation and sediment pollution in the lower Ebro River

Núria Cid[1,2], Carles Ibáñez[1], Rosa Andreu[1], and Narcís Prat[2]

[1] *Aquatic Ecosystems, IRTA, Carretera Poble Nou, Km 5.5, P.O.Box 200, E-43540 Sant Carles de la Ràpita, Catalonia, Spain.*

[2] *Departament d'Ecologia, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, E-08028 Barcelona, Catalonia, Spain.*

Corresponding author. Tel.: 0034- 93 40 11 88; Fax: 0034- 93 411 14 38 E-mail address: ncid@ub.edu

Abstract

Lower courses of large rivers receive the impact of multiple stressors. In this study, the hydrological conditions reflecting river regulation and the distance from the main dams together with the existence of a point source pollution of heavy metal and organochlorine compounds were considered to understand the temporal and spatial changes of the macroinvertebrate community and their biological traits. Furthermore, taxonomic and functional diversity metrics were used. The different hydrological situations in spring and autumn explained a higher variability of the biological traits compared to the taxonomic composition, which varied only slightly according to the temporal and spatial scales. While after an event of high flow the community was characterized by a major importance of resistance/resilience traits (high reproduction rate, higher mobility), after a period of constant flow the trait composition was more diverse and different strategies cohabited. Accordingly, functional diversity metrics were higher at stable flow conditions (Rao functional diversity). Despite of the high temporal variation of the biological traits, the trait composition changed in both studied seasons following a downstream gradient of decreasing impacts. Proportions of filter-feeders burrowing on the sediment and feeding on fine detritus increased with increasing distance from the source of impacts, while predators followed the opposite pattern. Also asexual reproduction was predominant in sites closer to the impact source. Along this impact gradient functional diversity metrics, including biological indeces, did not show a clear response, however in spring taxonomic metrics were higher at the lowermost sites due to the presence of relatively sensitive species. In general, our results showed that the different hydrologic conditions conditioned the other impacts such as pollution (i.e., heavy metal and organochlorine pollution) since the combined effect of a high water discharge with higher pollution exposures can occur due to the higher sediment remobilization.

Keywords: macroinvertebrates, biological traits, Ebro River, large rivers, functional diversity

1. Introduction

Human activities along the last century have lead to a progressive deterioration of freshwater ecosystems, by the alteration of natural processes, the overexploitation of resources and the pollutant loading. Large rivers, specially the lower courses, are a melting pot of stressor sources where hydrological alterations and land-uses coupled with pollution and the introduction of alien species are frequent and co-ocurring pressures affecting the river ecosystem (Petts et al., 1993; Poff et al., 2007; Klok & Kraak, 2008; Strayer et al., 2008).

Because benthic macroinvertebrates play a key role in aquatic ecosystems and respond considerably to environmental stressors and disturbances, they have been widely applied in the bioassessment of streams and rivers (Rosenberg & Resh, 1993) and integrated in the environmental policies as one of the most important organism groups to define their ecological integrity (European Commission, 2000). At community level, the taxonomic composition of macroinvertebrates has been the main approach for the biomonitoring of running waters by the use of many different metrics and indices, reviewed in Bonada et al. (2006), which is now fully implemented in all European and worldwide environmental policies, with a general use of the multimetric indices (Buffagni et al., 2006; Munné & Prat, 2009). More recently, studies assessing the effects of environmental stressors across lotic ecosystems have considered the use of several biological invertebrate traits (e.g. body size, reproduction, dispersal) as an alternative tool (e.g. Dolédec et al., 1999; Statzner et al., 2001; Statzner et al., 2005; Statzner and Bêche, 2010), assuming that changes in trait composition have consequences on ecosystem functioning (Statzner et al., 2004). This multitrait approach is based on the concepts of the River Habitat Templet (Townsend & Hildrew, 1994) which a priori predicts the species trait response to natural environmental disturbance, and set the basis to predict anthropogenic impacts in freshwaters (Bonada et al., 2006; Henle et al., 2006). Moreover, compared to the taxonomic approach, it presents a higher temporal and spatial stability across regions since the variability attributed to species composition of different areas is reduced to a several functional modalities (Statzner et al., 2004; Bêche et al., 2006; Bonada et al., 2007b). In the context of large rivers, although many different human impacts occur at the same time, the response of specific biological traits has been reported in Europe (Dolédec et al., 1999; Gayraud et al., 2003;

Bady et al., 2005; Statzner et al., 2005; Dolédec & Statzner 2008; Archaimbault et al., 2010).

According to the functional approach, the use of species traits has been incorporated in measures for the quantification of functional biodiversity in ecosystems (e.g. trait vs species richness, see Díaz & Cabido, 2001). In freshwaters, several studies have addressed simultaneously the taxonomic and functional diversity (Bady et al., 2005; Bonada et al., 2007a; Bêche & Resh, 2007; Statzner et al., 2007; Bêche & Statzner, 2009), obtaining different results depending on the study purpose. For example, Bady et al. (2005) obtained that functional diversity showed a higher accuracy with less sampling effort, and Bonada et al. (2007a) observed differences in functional diversity between the Mediterranean and temperate regions but not in taxonomic diversity. Therefore, including the measurement of functional diversity seems to be essential for an accurate assessment of the ecosystem.

As many of the large European rivers, the Ebro River is affected by multiple impacts coming from industrial, urban and agricultural activities (Lacorte et al., 2006; Terrado et al., 2006) and is strongly regulated by the construction of more than 190 dams which impound up to the 60% of the mean annual runoff for hydropower generation and irrigation purposes (Vericat & Batalla, 2006). The lower part of the river, considering the last 100 km from river mouth, is regulated by a complex of three large reservoirs that alter the sediment dynamics (Vericat & Batalla, 2006), the downstream flow and water temperature regimes and geochemistry (Ibáñez et al.,1995; Sabater et al., 2008; Prats et al., 2010). The proliferation of macrophyte beds throughout the lower part of river during the last 10 years is also of important concern since induces ecological changes that affect public health and economy in the area (Ibañez et al., 2008; Batalla et al., 2009). Moreover, this section of the river receives a permanent influx of heavy metals and organochlorine compounds originated from point source pollution due to the deposition of 300,000 t of industrial solid wastes located in the lowermost reservoir (Grimalt, 2006). These pollutants can be released downstream and thereby incorporated in the food web (Ramos et al 1999; Mañosa et al 2001; Cid et al., 2010; Suarez-Serrano et al., 2010; Barata et al., 2010) and cause potential sublethal effects (Bosch et al., 2009; Navarro et al., 2009). Finally, while populations of non-native species such as the zebra mussel (*Dreissena polymorpha*), the Asian clam (*Corbicula fluminea*) and the European catfish (*Silurus glanis*) are abundant and spread out in the area (Jimenez-Muer, 2001;

Sabater et al., 2008; Navarro et al., 2006; Carol et al., 2009), native species such as the freshwater mussel *Margaritifera auricularia* are endangered and populations decline (Araujo & Ramos, 2000). In this context, information on the macroinvertebrate community of the Ebro River is scarce, having only four published papers in the last fifteen years (see Gallardo et al., 2008, 2009a, 2009b, 2009c) and only two publications considering the lower course of the river (see Muñoz & Prat, 1994; Ibáñez et al., 1995). As far as we know, the only published macroinvertebrate data assessing the impacts in this part of the river is related with metal bioaccumulation of single species (Cid et al., 2010) or with endangered freshwater mussels (Altaba, 1990, 1997).

Because comparisons between structural and functional approaches applied to the same dataset are not abundant, the aim of this study was to compare the use of taxonomic and biological trait approaches to assess the community structure of the lower Ebro River at temporal and spatial-reach scales and how this is reflected in the metrics. The main objectives were: (1) to study whether different hydrological conditions caused by river regulation (spring vs autumn) determined the community taxonomic and trait composition, (2) to test whether macroinvertebrate assemblages and trait structure were randomly distributed or not along a spatial gradient, according to distance from the main origin of impacts, mainly the sediment contamination, and (3) to explicitly quantify the taxonomic diversity and functional diversity at both seasonal and reach scales and if this can be related to the effects of river regulation and the sediment contamination.

2. Material and Methods

2.1 Study area

The Ebro River is located in the NE Iberian Peninsula and has a drainage basin of 85,550 km^2 with a length of 928 km, being the largest river in Spain and one of the most important rivers discharging to the Mediterranean Sea. The lower part of the river (100 km from the river mouth) has a mean annual flow of 426 $m^3 \cdot s^{-1}$ (Ebro Water Authority, URL: http//: www.chEbro.es) and is regulated by two main hydropower dams constructed in the late 1960s: Mequinença with a capacity of 1534 hm^3, and Riba-Roja, with a capacity of 207 hm^3. The latter dam regulates the water flow of the Cinca and

Segre Rivers, the largest tributaries of the Ebro. Downstream of Riba-Roja the Flix Dam is the smallest one, with a capacity of 11 hm^3. Moreover, a nuclear power plant was built in 1984 in Ascó, 5 km downstream of the last dam. The study area is the freshwater part of the lower Ebro River, without the influence of the salt wedge (see Guillén et al., 1992; Ibáñez et al., 1997 for a detailed description of the area and its hydrological characteristics), extending from the Flix dam to Tortosa, downstream of the Xerta weir (Fig. 1). The riverbed is mainly composed of gravels, cobbles and sand (Muñoz and Prat, 1994; Batalla & Vericat, 2009).

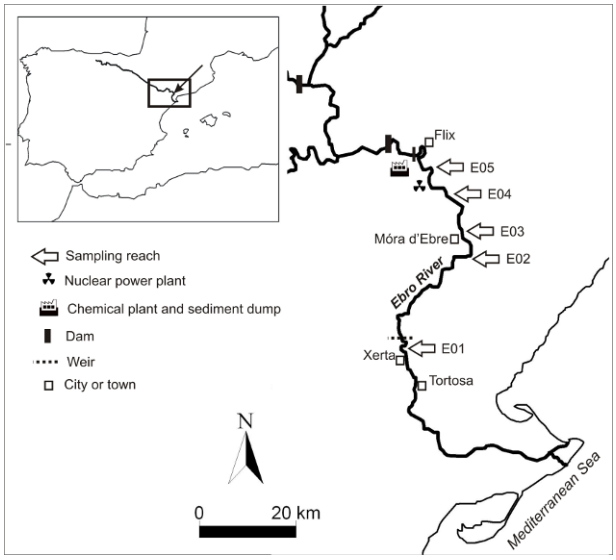


Fig.1 Sampling reaches in the lower Ebro River.

2.2 Sampling

Samples were taken in the end of May 2007, after the relative high flows of April, and in mid October 2007, after a period of low and constant flows during summer and beginning of autumn (Fig. 2).

In total, 5 reaches of 2 Km each were studied in order to have the highest representative variability of the system. The reaches were located along a spatial gradient downstream of the Riba-roja dam, where the effects of the dam regulation are originated and also downstream of the Flix dam where a large deposit of toxic sediments already exists. The main contaminants present in this sediment dump have been described in detail by Grimalt (2006). In previous studies (Cid et al., 2010) we described the effects of these contaminants in taxa present in the Ebro. As an example, data of the main pollutants

found in the caddisfly *Hydropsyche exocellata* are shown in Table 1, which clearly indicate the presence of both heavy metals and organochlorine compounds along the river. Since the present study aims to analyze the effects of contaminants at community level, Table 1 was not further analyzed along this paper.

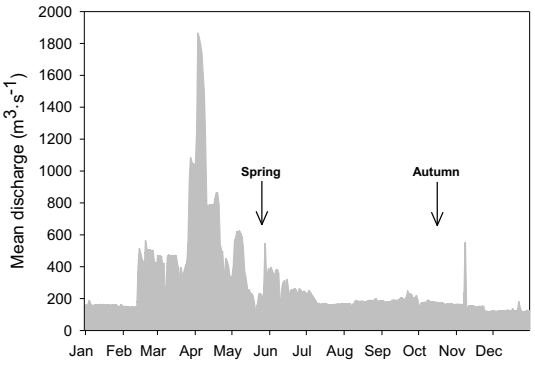


Fig.2 Mean daily discharge ($m^3 \cdot s^{-1}$) in the lower Ebro River below the dams for the year 2007. Arrows show the two sampling periods.

Table 1 Sampling reaches selected for the study.

Reach location	Reach code	Km[1]	Hg (µg· g^{-1})[2]	∑PCB (ng·g^{-1})[2]	∑DDT (ng·g^{-1})[2]
Flix	E05	3	0.93	800	222
Vinebre	E04	10	-	-	-
Móra	E03	21	0.46	338	116
Ginestar	E02	28	-	-	-
Xerta	E01	55	0.29	288	97

[1]River distance from the last dam and from the sediment dump.

[2]Concentrations in *Hydropsyche exocellata* (dry weight) from spring 2007. Hg data extracted from Cid et al., (2010) and PCB and DDT data from Cid et al., unpublished data (*in prep*).

Measurements of temperature, conductivity and oxygen were taken in the centre of each reach, since we considered that the reach was relatively homogeneous. The dissolved oxygen (DO) in water, conductivity, temperature was measured in situ with a YSI 556 multiprobe. Water samples for pigment, suspended organic matter and nutrients analysis were taken at 3 points across each reach (in the beginning, middle and in the end). For pigments and suspended organic matter, the methods of Steinman et al., (1996) for extraction were followed. The methods of Graffhoff et al., (1999) were used for the analysis of dissolved nitrites, nitrates, phosphates and Silicates.

The benthic macroinvertebrates were collected using a kicking net with a mesh size of 250 µm. The riverbed was disturbed and the subsequent sample was deposited in a tray in order to clean large cobbles (when present) of attached animals. In order to integrate all the habitat variability at each reach, benthic samples were composed by 5 different subsamples collected at different locations along a reach. Because the main channel was more than 2 m deep in all sampling sites, the sampled stations were limited to the wade able area (0-1 m). Afterwards invertebrate samples were preserved in 4% formaldehyde and taken to the laboratory to be identified. The identification of macroinvertebrates was done at the lowest possible taxonomic level.

2.3 Biological traits

Eleven biological traits containing 62 categories obtained from a published database (Tachet et al., 2000) were used to describe the macroinvertebrate community (Table 2). The traits in this database have an affinity score assigned for each taxa ranging from 0 to 5, from null affinity to high affinity, respectively (Chevenet et al., 1994)

When the taxonomic level of identification was higher than the level available for trait information in the database, the level present in the traits was assigned for that taxa. Because Oligochaeta were identified at a coarse level, the affinity scores were calculated by summing the affinity scores of the genera of this taxonomic group known in the Ebro and re-scaling the results to a 1–5 scale. When taxa identified were not present in the trait database they were excluded from the analysis, mostly microcrustaceans (Copepoda, Ostracoda, Cladocera) and early larval stages of Hydroptilidae and Coenagrionidae. Taxa representing only a 0.01 % of total abundance were omitted from the analysis (usually only one individual present in one subsample in one season).

2.4 Statistical analyses

For the analysis of the taxonomic community structure a log-transformed taxa-abundance dataset was used to perform a correspondence analysis (CA). In order to analyze the functional structure, a dataset of relative abundance of traits per sample was used to perform a fuzzy correspondence analysis (FCA) (Chevenet et al., 1994). In order to obtain the latter dataset, the affinity of each taxon for each trait category was multiplied by the taxon abundance (see Chevenet et al., 1994). In order to test the

statistical significance of CA and FCA, a random permutation test (Monte Carlo test, 1000 permutations) was used. Afterwards, Pearson correlation analysis was obtained in order to relate the physical and chemical environmental variables with the two axes of the CA and FCA and to detect any pattern according to the community distribution.

Table 2 Biological traits and categories for macroinvertebrates present in this study (see Tachet et al. (2000). Codes extracted from Mellado et al. (2008).

Biological traits	**Category**	**Code**
Maximal size	<0.25 cm	<0.25
	>0.25–0.5 cm	>0.25-0.5
	>0.5–1 cm	>0.5-1
	>1–2 cm	>1–2
	>2–4 cm	>2–4
	>4–8 cm	>4–8
Life cycle duration	<1 year	1
	>1 year	>1
Potential no. reproductive cycles per year	1	1
	>1	>1
Aquatic stages	Egg	egg
	Larva	lar
	Pupa	pu
	Adult	ad
Reproduction type	Ovoviviparity	ov
	Isolated eggs, free	efr
	Isolated eggs, cemented	ec
	Clutches, cemented or fixed	cfx
	Clutches, free	cfr
	Clutches, in vegetation	cv
	Clutches, terrestrial	ct
	Asexual reproduction	asx
Dispersal	Aquatic passive	aqp
	Aquatic active	aqa
	Aerial passive	aep
	Aerial active	aea
Resistance form	Eggs, statoblasts	ee
	Cocoons	co
	Cells against desiccation	cdes
	Diapause or dormancy	dia
	None	no
Respiration	Tegument	teg
	Gill	gi
	Plastron	plst
	Spiracle (aerial)	spi
Locomotion and substratum relation	Flier	fli
	Surface swimmer	sswim
	Full water swimmer	fswim
	Crawler	craw
	Burrower (epibenthic)	bur
	Interstitial (endobenthic)	int
	Temporarily attached	tatt
	Permanently attached	patt
Food	Fine sediment and microorganisms	s-m
	Detritus < 1 mm	fde
	Plant detritus > 1 mm	cde
	Living microphytes	lmph
	Living macrophytes	lMph
	Dead animal > 1 mm	dan
	Living microinvertebrates	lminv
	Living macroinvertebrates	lMinv
	Vertebrates	ver
Feeding habits	Absorber	ab
	Deposit feeder	depf
	Shredder	shr
	Scraper	scr
	Filter feeder	fil
	Piercer (plants or animals)	pier
	Predator (carver/engulfer/swallower)	pred
	Parasite	par

Pearson correlation analysis was also made with the relative abundance of trait categories along the first two axes of the fuzzy correspondence analysis in order to test significant changes trait categories.

To calculate explicit taxonomic and functional measures of the community, the taxon and trait richness and the Shanon-Wiever, Simpson and Rao indices were calculated for the genus and trait relative abundance per sample. The Rao index (Champely & Chessel, 2002; Bady et al., 2005; Bonada et al., 2007a) is calculated by using the dissimilarity matrix (measured, for instance, as Euclidean distance) of the dataset of relative abundance of species or traits per sample (see above explanation for this dataset). Nonparametric Kruskal-Wallis tests were performed to detect seasonal differences. Moreover, we calculated the IBMWP biological index (Alba-Tercedor & Sánchez-Ortega, 1988; Alba-Tercedor et al., 2004) to assess the biological quality of the Ebro, which is the most used biological index in Spain and has been recently intercalibrated with the Intercalibration Common Metric index (ICMi) (see Munné & Prat, 2009).

All the statistical multivariate analysis and several graphs were carried out using R free software (Ihaka & Gentleman, 1996) with ADE4 (Thioulouse et al., 1997) and Vegan (Oksanen et al., 2010) libraries. Sigma Plot (Systat Software Inc.) was used as complementary software for graphics.

3. Results

3.1 Taxonomic and trait composition of the macroinvertebrate community

The lower Ebro's zoobenthos was mainly dominated by oligochaetes, chironomids (Orthocladiinae), amphipods, Nemertea (*Prostoma graecense*) and by the invasive asian clam *Corbicula fluminea* (Fig. 3). Other taxa such as the Turbellaria and Hydrozoa were also very abundant. Ephemeroptera (*Baetis* sp., *Caenis luctuosa*, *Ephoron virgo*) and Trichoptera (*Hydroptila* sp., *Hydropsyche exocellata)* can be also relatively abundant, but not at the high densities as the former ones. The community composition is similar to those described by Muñoz and Prat (1994) with the exception of the introduced alien species such as *Corbicula fluminea*, absent previous to our study, which has become a dominant species.

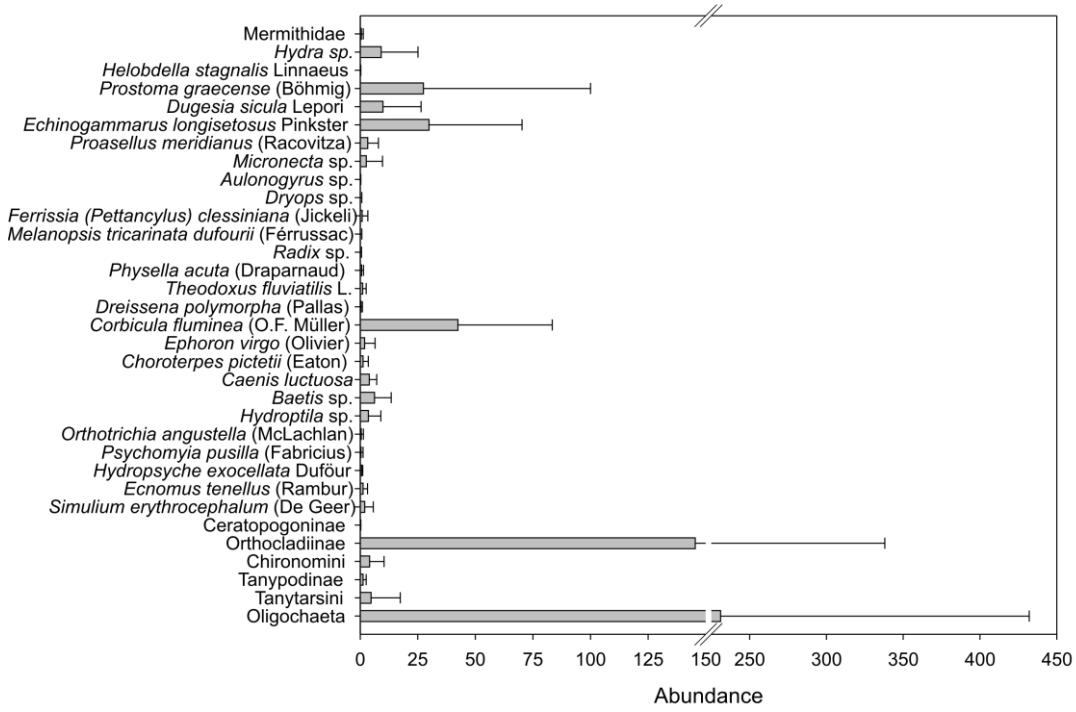


Fig. 3 Mean abundance and standard deviation of each taxa in the lower Ebro River in 2007.

The functional structure of the benthic community was dominated by taxa with a size between 0.5-1 cm, with a lifespan less than one year and with multivoltine life cycle (Fig. 4). Reproduction was mainly by clutches cemented or fixed and by ovoviviparity, and the main dispersal method was aquatic passive. Most of the taxa had no resistance forms and respiration mode was by tegument and gills. Those having aquatic stages as larvae and egg were predominant, and the most frequent locomotion modes were crawling, swimming and burrowing. Filter-feeders and deposit feeders were the most abundant and the main food sources were fine detritus particles (<1mm) and living microphytes as diatoms. Within the traits, feeding habits and food were correlated with the number of reproduction cycles per year, locomotion was correlated with resistance form, and aquatic stages were correlated with life cycle duration. The other traits analyzed in this study (dispersal, reproduction, maximal size and respiration) presented more independence (Fig. 4, circle).

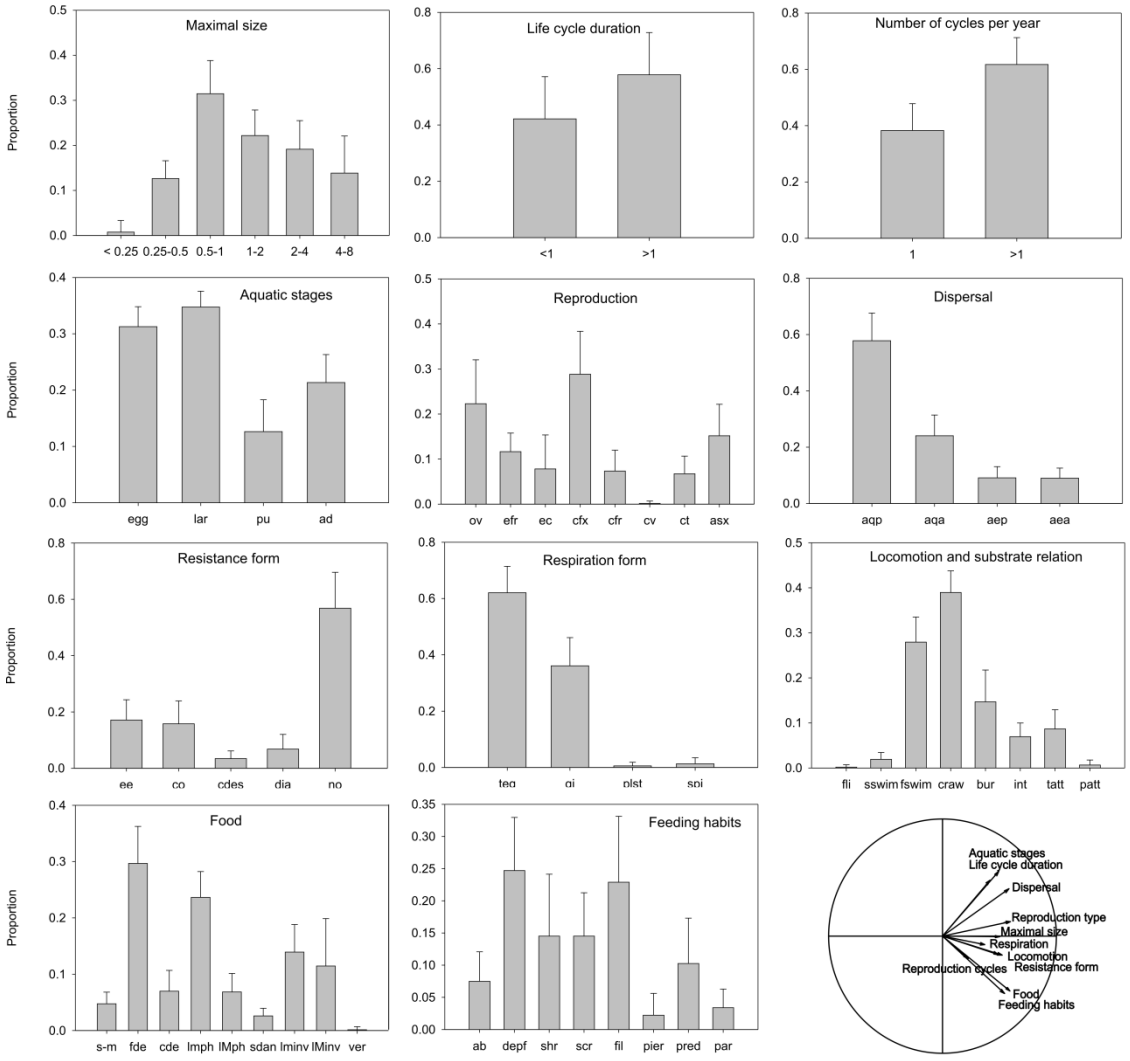


Fig. 4 Mean proportion and standard deviation of each trait category in the lower Ebro River weighted by taxon abundance and correlation circle illustrating the relationship among biological traits. See table 2 for category codes.

3.2 Structural and functional variability of the macroinvertebrate community

Both the CA based on taxonomy and the FCA performed with relative abundances of the biological trait categories revealed a temporal effect (along the first axis) and a gradual spatial effect with increasing distance downstream of the dams (on the second axis) (Fig. 5). Both analysis were statistically significant (Monte Carlo test, $P<0.001$). For the CA based on the genus abundances, the first and second axes explained 28.9% and 24 % of the total variability, respectively (Fig. 5a). In this analysis, once the spring and autumn samples were delimited, the latter presented a strong separation of the first two reaches below the dams (E05 and E04) from those more downstream (E03, E02 and E01). For the FCA performed with traits, the explained variability was much higher

than in the CA since the first and second axes explained 50.3% and 22.8%, respectively (Fig. 5b). Here, the longitudinal change of the community according to the functional structure was more gradual.

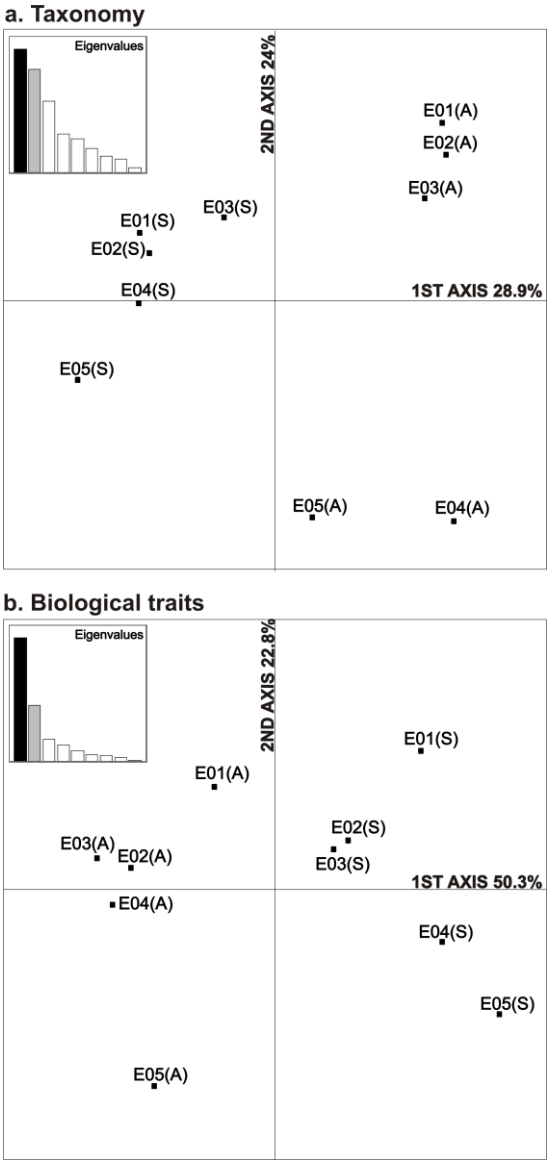


Fig. 5 Results of the Correspondance Analysis (CA) performed on the relative abundance of the species (a), and of the Fuzzy Correspondence Analysis (FCA) performed on the relative abundance of the biological trait categories of macroinvertebrates (b). E05 is the reach located a few km downstream of the dam and E01 the one more downstream (see Table 1). Label A and S in reaches are Autumn and Spring, respectively.

The correlation analyses of the environmental variables with the first two axes of the CA (taxonomy) and of the FCA (biological traits) are shown in Table 3. Since the first axis of both analysis were related to temporal changes, they showed significant

correlation with water temperature, pH, conductivity, salinity, phosphates ($P\text{-}PO_4$), total dissolved phosphorous (TDP), total dissolved nitrogen (TDN) and silicates (Si), having all variables higher values in autumn. Moreover, the first axis of the FCA showed a correlation with the concentration of phaeopigments, also with higher concentrations in autumn. The spatial change of the community structure observed along the second axis of both ordinations was significantly correlated with river distance, with higher factorial scores in the reaches located more downstream (Fig. 6). Within the physic and chemical variables, only chlorophyll (chl *a*) and nitrites ($N\text{-}NO_2$) showed a significant correlation (decreasing concentrations along the second axis), having higher concentrations in the reaches closer to the dam.

Table 3 Pearson correlation analysis of environmental variables and the first two axis of the correspondence analysis (CA) based on genus abundances and of the fuzzy correspondence analysis (FCA) based on biological traits. The level of significance is indicated with * ($P<0.05$) or** ($P<0.01$) or ***($P<0.001$).

	Taxonomy		**Biological traits**	
Variables	**1st AXIS**	**2nd AXIS**	**1st AXIS**	**2nd AXIS**
Km	0.14	0.72*	0.02	0.88***
U (m/s)	-0.26	0.27	0.29	0.25
T^a (ºC)	0.93***	0.00	-0.91***	0.03
pH	0.88***	-0.18	-0.83**	-0.24
Cond (µs/s)	0.94***	-0.09	-0.94***	-0.09
Salinity (ppt)	0.93***	-0.09	-0.93***	-0.09
DO (mg/l)	-0.51	0.04	0.54	0.30
DO (%)	-0.16	0.04	0.20	0.33
SPM (mg/l)	-0.10	-0.16	-0.10	-0.02
OM (mg/l)	-0.01	0.18	0.00	-0.18
OM (%)	0.02	0.35	0.16	-0.16
Chl a (µg/l)	0.32	-0.74*	-0.41	-0.67*
Phaeo (µg/l)	0.47	-0.62	-0.66*	-0.54
$P\text{-}PO_4$	0.88***	-0.26	-0.96***	-0.20
TDP	0.89***	-0.15	-0.90***	-0.13
$N\text{-}NH_4$	0.57	-0.08	-0.44	-0.21
$N\text{-}NO_2$	-0.47	-0.77**	0.30	-0.85**
$N\text{-}NO_3$	0.71*	-0.22	-0.84**	-0.07
TDN	0.84**	-0.13	-0.89***	-0.03
Si	-0.74*	0.08	0.76*	0.35

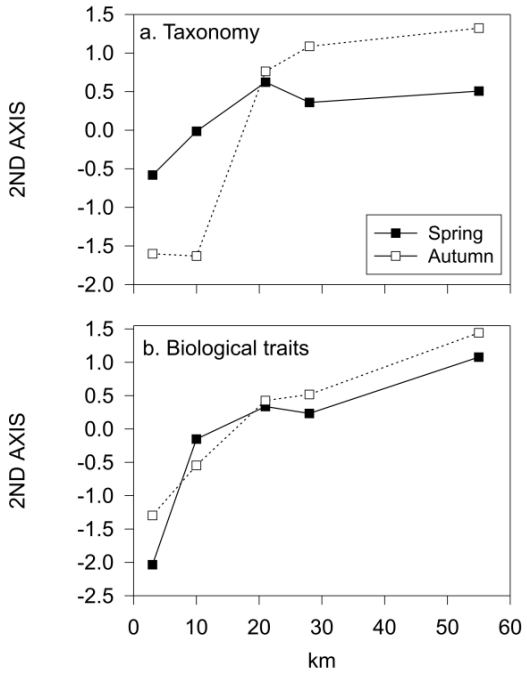


Fig. 6 Factorial scores of the second axis of the correspondance analysis (CA) on the relative abundance of taxa(a), and of the fuzzy correspondence analysis (FCA) on the relative abundance of trait categories (b) plotted against river distance downstream of the dam and sediment dump. See Table 1 for locate stations at river km.

The seasonal and spatial patterns of taxa are shown along the first and second axis of the CA in Fig. 7. The first axis showed a gradient starting from species more abundant in spring (e. g. the crustaceans *Echinogammarus longisetosus* and *Proasellus meridianus* and the Orthocladiinae) or only present in spring (e.g. the Ephemeroptera *Ephoron virgo* and *Choroterpes pictetii*) from those predominant in autumn (e.g. *Simulium erythrocephalum*, *Prostoma graecense* and *Dugesia sicula*). Permanently abundant taxa along both seasons were the Oligochaeta, *Caenis luctuosa*, *Corbicula fluminea*, *Theodoxus fluviatilis*, and *Hydropsyche exocellata*, among others. The longitudinal pattern along the second axis of the CA separated those species in the first reaches close to the dam (left part of the graph) from those located more downstream (Fig. 7b). As the river km increased from the dam, the number of Ephemeroptera taxa was higher, as *Ephoron virgo*, *Choroterpes pictetii* and *Baetis* sp. while crustaceans as *Proasellus meridianus*, the chironomids Tanypodinae and Tanytarsini and the Trichoptera *Ecnomus tenellus* were more abundant in the first km. The gastropod *Melanopsis tricarinata* was exclusive in the last reach located 40 km from the dam (E01). Within the invasive species, the zebra mussel (*Dreissena polymorpha*) was predominant in the

first reaches close to the dam while the Asian clam (*Corbicula fluminea*) increased the abundances with increasing distance from the dam.

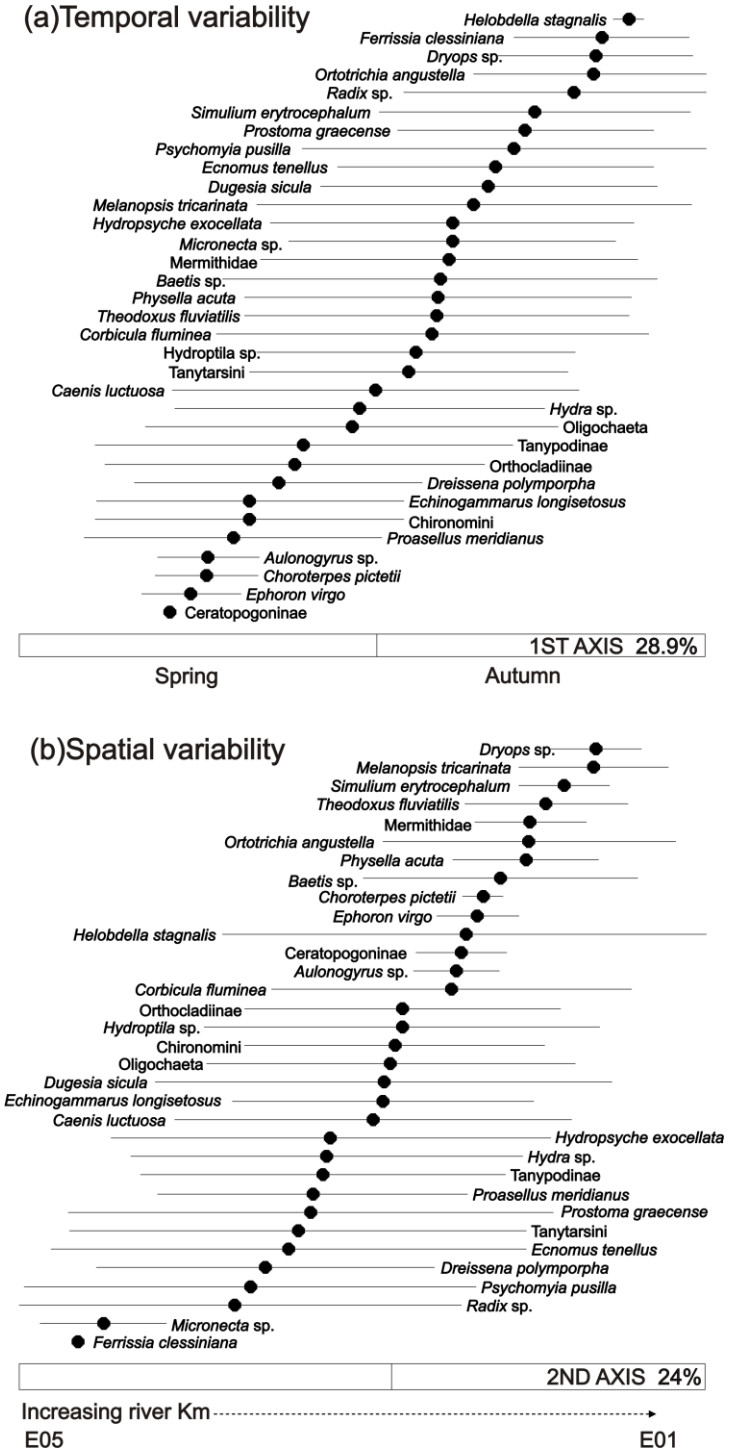


Fig. 7 Position of taxa on the first axis (a) and second axis (b) of the correspondence analysis using taxonomy. The position of each taxa (dots) corresponds to the weighted mean of their distribution in sites and the horizontal lines are the standard deviation. The first axis represents the taxa distribution according to seasonality and the second according to the longitudinal gradient.

The correlation ratios of the biological traits with the first two axes of the fuzzy correspondence analysis explain the contribution of each trait to the temporal and spatial variability observed (Table 4). The first axis (temporal) was mainly explained by changes in the feeding habits, reproduction, food, respiration and dispersal. Many of these categories also contributed to the variability on the second axis and therefore to the longitudinal patterns, as feeding habits, reproduction and food. On the contrary, the aquatic stages and life cycle duration were the traits with a lowest contribution to the variability of the analysis.

The macroinvertebrate community in spring was characterized by significantly higher abundances of shredders (Table 5), occupying the positive side of the first axis, while absorbers and piercers were significantly more abundant in autumn, occupying the negative side also with predators (Fig. 8). The proportions of filter-feeders, deposit feeders and scrapers were more homogeneous along both seasons since they are the main feeding strategies in the community. The macroinvertebrates in spring had plant detritus >1 mm and living microphytes as the distinctive food sources, while in autumn the living macroinvertebrates were predominant, thus a high proportion of predators was present. The characteristic reproduction type in spring was ovoviviparity and clutches (free or terrestrial) and an aquatic passive dispersal while in autumn the reproduction was by clutches in vegetation or clutches cemented and the dispersal was aquatic active. The other traits (maximal size, reproductive cycles per year, locomotion, resistance form, aquatic stages and life cycle duration) did not largely contribute to explain the variability, however they also inform about the characteristics of the community at each season.

Table 4 Correlation coefficients of biological traits with the first two axes of the fuzzy correspondence analysis.

Biological trait	1ST AXIS	2ND AXIS
Feeding habits	0.0580	0.0274
Reproduction	0.0532	0.0147
Food	0.0420	0.0110
Respiration	0.0301	0.0036
Dispersal	0.0230	0.0044
Maximal size	0.0212	0.0242
Reproduction cycles/year	0.0163	0.0008
Locomotion	0.0128	0.0166
Resistance form	0.0120	0.0169
Aquatic stages	0.0077	0.0020
Life cycle duration	0.0001	0.0039

Table 5. Pearson correlation analysis of biological traits and the first two axis of the fuzzy correspondence analysis (FCA). The level of significance is indicated with * (P<0.05), ** (P<0.01) or ***(P<0.005). The first axis is related with temporal variability and the second axis with the longitudinal gradient across reaches (see Fig. 7).

Biological trait	Category	1st AXIS	2nd AXIS
Maximal size	<0.25 cm	-0.31	-0.74**
	>0.25–0.5 cm	-0.76**	-0.12
	>0.5–1 cm	0.87***	-0.03
	>1–2 cm	-0.62	0.32
	>2–4 cm	0.87***	-0.43
	>4–8 cm	-0.33	0.67*
Life cycle duration	≤1 year	0.12	0.63*
	>1 year	-0.12	-0.63*
Potential no. reproductive cycles per year	1	-0.92***	0.20
	>1	0.92***	-0.20
Aquatic stages	Egg	-0.83**	0.36
	Larva	0.79**	0.37
	Pupa	0.71*	-0.28
	Adult	-0.62*	-0.47
Reproduction type	Ovoviviparity	0.86**	-0.32
	Isolated eggs, free	0.29	0.85**
	Isolated eggs, cemented	-0.39	0.48
	Clutches, cemented or fixed	-0.96***	-0.03
	Clutches, free	0.73*	-0.31
	Clutches, in vegetation	-0.45	0.41
	Clutches, terrestrial	0.80***	0.11
	Asexual reproduction	-0.32	-0.68*
Dispersal	Aquatic passive	0.87***	-0.30
	Aquatic active	-0.98***	-0.03
	Aerial passive	-0.09	0.35
	Aerial active	0.16	0.90***
Resistance form	Eggs, statoblasts	0.31	0.85***
	Cocoons	-0.89***	-0.17
	Cells against desiccation	-0.41	0.67*
	Diapause or dormancy	-0.28	-0.24
	None	0.53	-0.67*
Respiration	Tegument	-0.91***	-0.18
	Gill	0.94***	0.21
	Plastron	-0.56	-0.59
	Spiracle (aerial)	-0.79**	-0.02
Locomotion and substratum relation	Flier	-0.41	0.44
	Surface swimmer	-0.55	-0.49
	Full water swimmer	-0.35	-0.73*
	Crawler	0.64*	0.18
	Burrower (epibenthic)	-0.03	0.85***
	Interstitial (endobenthic)	0.92***	-0.34
	Temporarily attached	-0.62*	-0.12
	Permanently attached	0.06	-0.73*
Food	Fine sediment and microorganisms	0.45	-0.04
	Detritus < 1 mm	0.62*	0.62*
	Plant detritus > 1 mm	0.82**	-0.36
	Living microphytes	0.60	0.58
	Living macrophytes	-0.02	-0.04
	Dead animal > 1 mm	-0.41	-0.45
	Living microinvertebrates	-0.10	-0.73*
	Living macroinvertebrates	-0.94***	-0.17
	Vertebrates	-0.37	0.36
Feeding habits	Absorber	-0.88***	-0.34
	Deposit feeder	0.59	0.40
	Shredder	0.85***	-0.51
	Scraper	-0.22	0.64*
	Filter feeder	-0.42	0.77**
	Piercer (plants or animals)	-0.83***	-0.20
	Predator (carver/engulfer/swallower)	-0.60	-0.68*
	Parasite	0.421	0.35

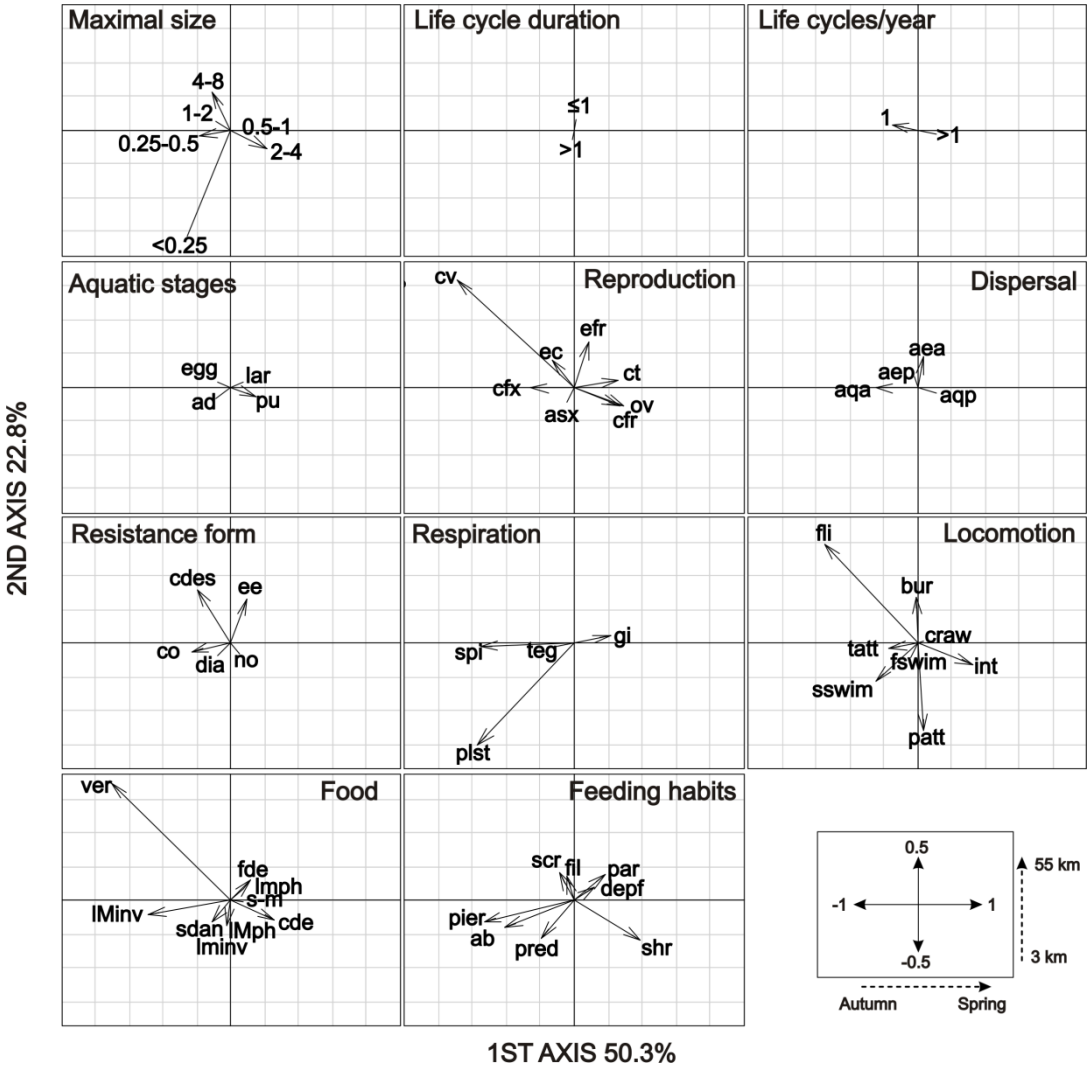


Fig. 8 Distribution of the biological trait categories on the first two axes of the fuzzy correspondence analysis (FCA) including both seasons. (See Table X for trait category codes).

Along the second axis (longitudinal gradient), shredders and predators were in the negative side and corresponding to the community in the first reaches (E05 and E04), while filter-feeders, scrapers and parasites were on the positive side and thereby more abundant starting from the third reach (E03) located 21 km downstream. Locomotion also created a gradient along this axis, with a higher proportion of organisms permanently attached to the substrate or swimming in the water surface in the first reaches and a higher proportion of burrowers and fliers with increasing river distance to the dams. The macroinvertebrates with cells against desiccation and eggs as resistance form were positioned in positive part of the first axis, thus being present in the more downstream reaches. On the other hand, those not having any resistance form, diapauses

or cocoons were mainly present in the first reaches below the dam (negative part of the second FCA axis). Organisms reproducing by ovoviviparity and by free clutches were predominant in reaches close to the dam while the opposite pattern was observed for those reproducing by clutches in vegetation or by isolated eggs (either free or cemented eggs).

3.3 Taxonomic and functional-based community metrics

Although most of the community (richness, Simpson's diversity, Shannon's diversity) and some trait metrics (trait Simpson's diversity and Rao's diversity) did not show any significant difference between spring and autumn, trait richness, trait Shannon's diversity and trait Rao's diversity were significantly higher in October (Fig. 9). In the case of richness, a similar number of taxon between seasons resulted in higher functional richness in autumn. On the other hand, slightly higher taxonomic diversity resulted in significant differences for functional diversity (Shannon's and Rao's indexes). The functional Simpson's diversity did not show a significant seasonality, although its values were also higher in autumn, probably because this index gives more relevance to the evenness of traits than to the trait richness.

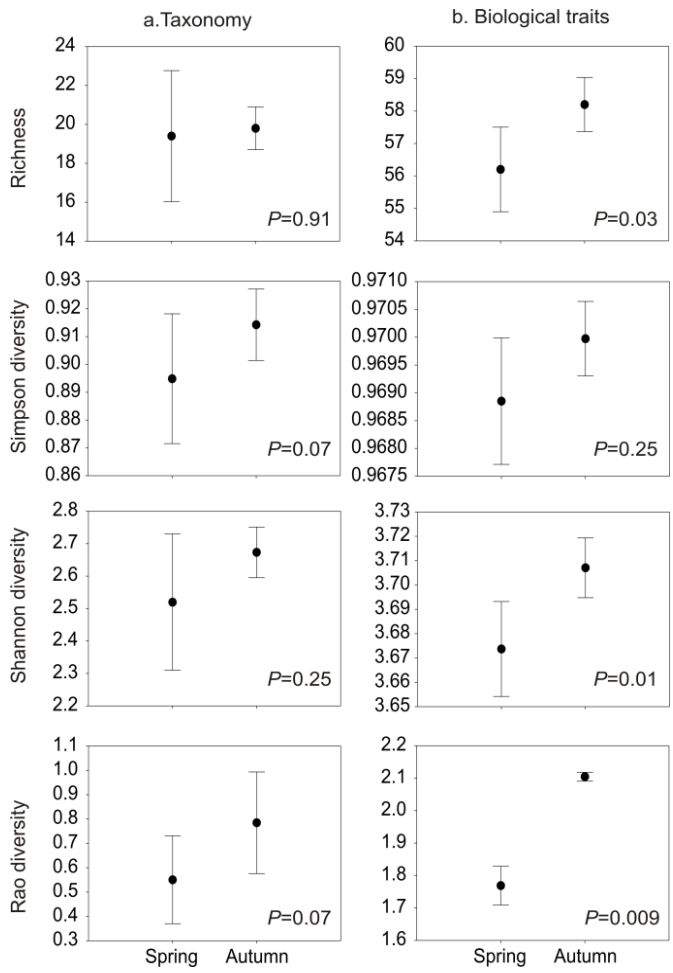


Fig. 9 Mean and standard deviation of richness, Simpson's diversity, Shannon's diversity and Rao's diversity in spring and autumn based on taxonomy(a) and with biological traits (b). *P*-values from Kruskal-Wallis test are indicated.

When assessing the longitudinal gradient as a function to the distance to the source of impacts, different patterns were obtained for every sampling period. In spring, a trend of increasing values of taxonomic metrics (richness, Simpson's, Shannon's, Rao's diversity) was observed with increased distance downstream of the dam (Fig. 10). Functional metrics showed less variability with distance from the source of impacts (e.g. trait Rao diversity), although Simpson and Shannon appeared to slightly increase. In autumn, after a period of constant flows, the longitudinal patterns were less variable and had higher values than in spring, according to the observed temporality.

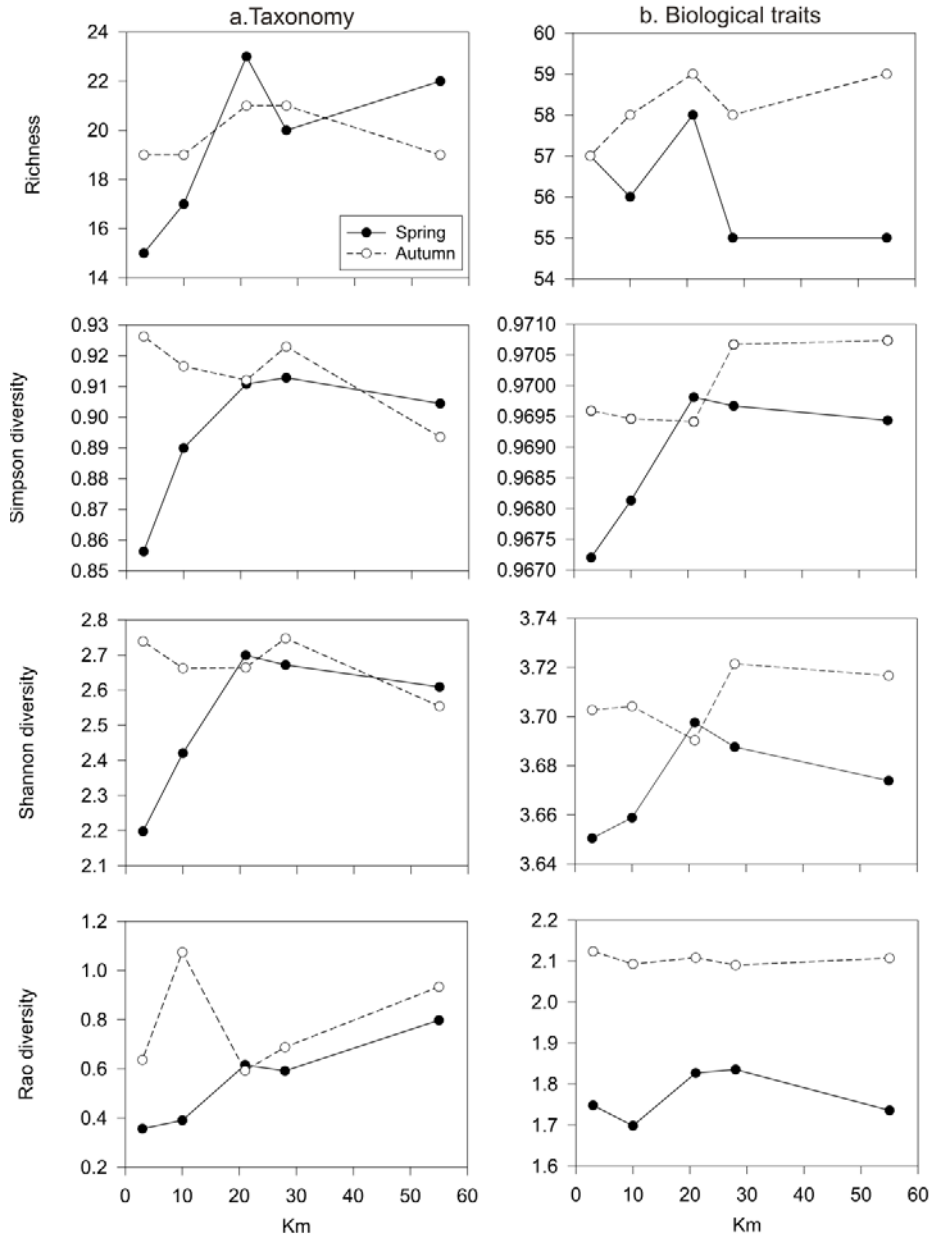


Fig. 10 Richness, Simpson's diversity, Shannon's diversity and Rao's diversity along river km downstream of the dam in spring and autumn based on taxonomy (a) and with biological traits (b).

From the point of view of biotic indices, the IBMWP index followed a similar pattern than the taxonomic richness, either in every season or along the longitudinal gradient (Fig. 11). According to this index and considering the fluvial type (i.e., main courses of rivers), the biological quality of the lower part of river was good either in spring or autumn. However, when assessing the quality considering the distance from the main dams, the first 10 km have a lower quality than the following reaches located more downstream (more than 20 km). Most of the taxa found are highly tolerant to pollution

and the relatively high values of the IBMWP index were attributed to the high community richness. This is clearly seen in Fig. 12, where the proportion of macroinvertebrates at each category of the IBMWP is showed. Only very few intolerant taxa (scores 7-10) are present while a relatively high proportion of taxa are not included in the index (e.g., species as *Corbicula fluminea* and some native species as *Melanopsis tricarinata)*.

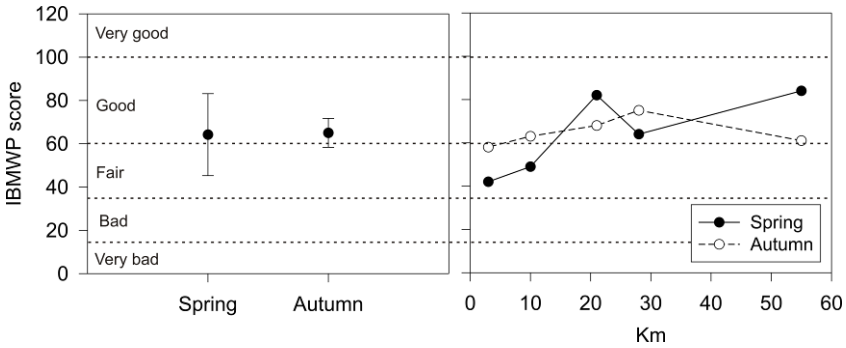


Fig. 11 Mean value and standard deviation of the IBMWP index in spring and autumn and along river km downstream of the dam.

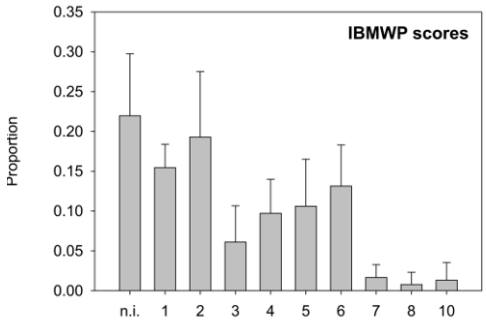


Fig. 12 Mean value and standard deviation of each IBMWP score in the lower Ebro River macroinvertebrate community weighted by taxon abundance. n.i.= taxa not included in the index IBMWP. The higher the IBMWP scores the more sensitive are the species to pollution.

4. Discussion

4.1 Importance of temporal patterns at different hydrological conditions

In the lower Ebro River, the biological trait composition was determined by the temporal variability, as well as in the analysis based on the taxonomic composition, although in the latter the difference was not so marked. This evidences the importance of temporal changes in communities whose biological traits are favored under certain

environmental conditions (Southwood, 1988; Townsend and Hildrew, 1994). For instance, in Mediterranean rivers the temporal heterogeneity structuring benthic communities due to seasonal changes related with discharge fluctuations is well described (Gasith & Resh, 1999; Bêche et al., 2007; Bonada et al., 2007b; Puntí et al., 2009). However, although located in the Mediterranean region, the Ebro is a highly regulated river as the flow variability is determined by the flow released from dams, and the frequency, timing and duration of flow related events are altered as in many other regulated rivers (Poff et al., 1997b).

In this study, there was a relatively high flow period in April previous to our spring samplings which could generate a medium habitat disturbance compared with the floods present in the Ebro before the dam construction, while the constant low flow observed from June to October lead to a stabilization of habitat conditions and an increase in dissolved nutrients and salinity. After a disturbance, inhabiting organisms should be those exhibiting more resilience or resistance (Townsend & Hildrew, 1994), i.e. those with an ability to recolonize or those with and ability to persist. Accordingly, most of the taxa that colonized the habitat in spring predominantly presented several reproduction cycles per year, linked with their fast development and population growth (e.g. Orthocladiinae). In the same theoretical templet, small body size and short lifespan should be expected. However, inconsistencies were found in crustaceans with a lifespan more than one year and a size up to 4 mm (*E. longisetosus*) or Ephemeroptera with one reproductive life cycle per year and a maximal size of 1-2 cm (*E. virgo*). These controversy has been previously observed in other studies including highly diverse communities and it has been attributed to trade-offs between traits (Resh et al., 1994; Statzner et al., 1997). Moreover, traits related with life cycle seem to be determined by long term adaptation to predictive natural flow events under certain climatic conditions (Williams, 1996; Lytle & Poff, 2004). On the other hand, the univoltine species which their life cycle is synchronized with water temperatures are important in the lower Ebro (Cid et al., 2008). Thus, it is important to distinguish which traits can be favored or disfavored by local discharge events and which traits might be independent of those short-term habitat fluctuations. For instance, locomotion is considered one of the traits more dependent on habitat characteristics (Williams, 1996; Bonada et al., 2007b). Highly mobile organisms have been predicted to adapt to habitats under high temporal variability (Mackay, 1992; Townsend & Hildrew, 1994) which can be related with the

refugia availability (Gjerlov et al., 2003). In the lower Ebro, the proportion of highly mobile organisms in spring was important and was linked with high abundances of crawlers with the ability to move into the hyporreos (e.g. Orthocladiinae, *Proasellus meridianus*).

In autumn, the stable habitat conditions propitiated a community with a wider variety of traits, since those trait categories favored by the stable conditions cohabited with those adapted to more unstable habitats (Usseglio-Polatera, 1994; Townsend et al., 1997). For instance, swimmers and fliers (e.g. *Dryops* sp.) cohabited with those attached to the substrate (e.g. *Simulium erythrocephalum*). The predominance of shredders in spring moved to a widening of the feeding strategies in autumn, as absorbers, piercers, predators, filter-feeders and scrapers cohabited as a result of a higher competition and specialization (Southwood, 1988; Townsend & Hildrew, 1994; Useglio-Polatera, 1994). Thus, the higher habitat heterogeneity (patchiness) due to a combination of different hydraulic condition provided a higher combination of traits (Townsend et al., 1989; Bonada et al., 2007b). Furthermore, an excessive proliferation of periphyton is common in regulated rivers, being enhanced under low flow conditions (Biggs, 2000). The low flows together with the higher nutrient concentration in autumn might have enhanced the periphyton growth since many piercers attached to substrate such as hydroptilid caddisflies (*Orthotrichia angustella*, *Hydroptila* sp.) and scrapers as snails (*Melanopsis tricarinata*, *Theodoxus fluvitilis*) were more abundant than in spring. Similar patterns were observed by Suren & Jowett (2006) after a period of low flows in a New Zealand gravel bed rivers under regulation. Those combination of environmental factors also favor the proliferation of rooted macrophytes (i.e. *Potamogeton pectinatus*), which, in turn, change the hydraulic conditions in the habitat they occupy and contribute to its homogenization (Batalla & Vericat, 2009). Moreover, we should consider that if a low flow situation prevailed throughout the year the effects to the ecosystem could be critical and different results might be obtained. Thereby, the combination of medium-sized flows in spring and the following stabilization until the beginning of autumn determined a community with more traits.

The observed temporal change in some of the biological trait metrics reflected a change in the taxonomic composition, since similar number of genus present in autumn proportioned a higher functional structure. The effects on functional diversity by habitat change due to flow variability had been reported in US Mediterranean streams (Bêche

& Resh, 2007), where a negative relationship of functional diversity (measured with Simpson's and Rao's indexes) with increasing flow was found in perennial streams. Both results are in agreement with the theoretical relation between functional diversity and environmental constraints proposed by Mouillot et al. (2006) for fish assemblages, where functional diversity decreases with increasing environmental stress. On the other hand, another explanation for the lower trait richness and diversity in spring could be obtained because those species exclusive in this period did not contribute to a higher functional diversity, since they might present similar traits as those present during both seasons. For instance, regarding the feeding habits, *Ephoron virgo* which is only present in spring has the same feeding strategy as a filter feeder than the alien *Corbicula fluminea* which was very abundant either in spring or autumn. Thus, it might be possible that different patterns were obtained if alien species were not present in the area or not considered in the analysis (see Gayraud et al., 2005).

4.2 Importance of the longitudinal changes

Several studies have applied the trait response to detect human impacts as a measure of ecosystem functioning at many scales (Dolédec et al., 1999; Charvet et al., 2000; Statzner et al., 2001; Gayraud et al., 2003; Usseglio-Polatera & Biesel, 2002; Lecerf et al., 2006, Tomanova et al., 2008; Dolédec & Statzner, 2008; Péru & Dolédec, 2010; Archaimbault et al., 2010) even if the same traitsdid not present the same importance among studies. For instance, life cycle duration, body form, reproduction and number of descendants per life cycle were the traits presenting more variability with increasing human impact in the Rhone River (Dolédec et al., 1999), while in the context of large European rivers other traits such as the feeding habits, food and maximal size were determinant (Gayraud et al., 2003). This inconsistency on the trait response might be a result of the different kind of impacts and combination of them which affect differently some traits or others (Dolédec & Statzner, 2008). In our study area, the main human impacts are derived from river regulation and heavy metal and organochlorine pollution. Thus, since the effects of discharge variation have been discussed in the previous section, the effect of the main dams including the presence of heavy metal and organochlorine contaminants in the lowermost reservoir was considered for the discussion according to a spatial gradient. Possible differences due to mesohabitat conditions at each reach were discarded since samples integrated several sites within a reach and no longitudinal differences were observed according to water velocity.

The results of Dolédec & Statzner (2008) according to the trait response to heavy metal pollution in large European Rivers demonstrated that within the trait categories they selected (i.e. maximal size ≤ 1 cm, animal food of all size and gill respiration) only the proportion of small organisms decreased with increasing heavy metal impact. Their *a priori* predictions assumed that for small organisms (with large surface-volume ratios) the external contact with metals could be critical. Thus, the way of exposure to heavy metals should determine the specific response of traits. In our study area, the heavy metal pollution is mainly originated by the release of pollutant loading from the sediment dump in the Flix dam, and the ingestion of particulate matter has been suggested as the main way of exposure in macroinvertebrates (Cid et al., 2010). For that reason, we considered that feeding habits and food (both traits correlated) should be the traits with a higher response to increasing distance from the sediment dump. Concretely, the proportion of filter-feeders feeding on detritus < 1mm where pollutants might be adsorbed appeared to be lower in the first reaches close to the impact (Fig. 13). Moreover, Dolédec & Statzner (2008) pointed out that the biotic interactions might increase the complexity of trait response to this type of stress since metal pollution can increase predation activity (Clements, 1999; Pollard & Yuan, 2006), although De Lange et al. (2004) obtained that predation was independent of sediment contamination. In our case, a higher abundance of predators was observed in the first reaches downstream of the sediment dump (Fig. 13). Recently, Archaimbault et al. (2010) obtained a high response in feeding habits and food, dispersal, reproduction and respiration when assessing sediment pollution containing either heavy metals or organochlorine compounds in French streams. In agreement with their results, our study also found a higher proportion of invertebrates with asexual reproduction and feeding on microinvertebrates in the more impacted sites and more scrapers and aerial active dispersal in the less impacted sites. On the contrary, they observed an increase in filter-feeders in impaired sites and obtained no differences for those feeding on fine detritus. Moreover, we did not found any significant response in any modality of respiration.

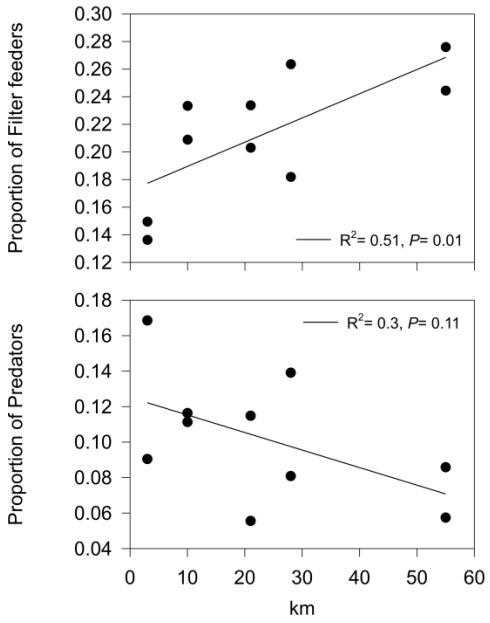


Fig. 13 Relationship between the proportion of filter feeders and predators with increasing distance from the source of impacts. Linear regressions are shown.

These differences can be due to the river size studied, since different responses can be expected when comparing effects of pollutants in medium-size streams or large rivers, according to their different ecological processes and dynamics. Thus, although relative abundance of biological traits discriminated between levels of contamination (Archaimbault et al., 2010), it is difficult and complex to obtain a mechanistic explanation based on a priori predictions (Dolédec & Statzner, 2008). Because other ecophysiological traits linked to phylogeny and evolution are involved in the ability of insects to deal with metal pollution (Buchwalter et al., 2008), the response of some biological traits could be weak. Poff et al. (2006) suggested that one of the main requisites for the selection of appropriate traits to detect environmental impacts was their phylogenetic independence. Therefore, the role of biological traits could be difficult to interpret since only those taxa with high elimination rates and/or high detoxification capacity can thrive in metal polluted areas. As an example in the present study, the metal tolerant *Hydropsyche exocellata* (Solà et al., 2004) was distributed along all reaches while the pollutant-sensitive *E. virgo* (De Haas et al., 2002) had the highest densities in the lowermost and less impacted area. Thus, the assessment of pollutant-enriched freshwaters should be also addressed with other approaches widely used in this field such as bioaccumulation, biomarkers or bioassays using keystone species (Bonada et al., 2006). In another perspective, the quantification of several traits

such as dispersal (e.g. aerial dispersal can transfer pollutants to the terrestrial foodweb), aquatic stages (e.g. the pupae can be more susceptible to predation by fish), feeding habits (e.g. filter feeders can incorporate pollutants in the particulate matter) and respiration (e.g. gill respiration can be critical when dissolved metals are present) can be useful tools for the application of accurate ecological risk assessment studies and for the selection of bioassay organisms in areas with high pollutant loading to the ecosystem (Ducrot et al., 2005).

The community metrics along the spatial gradient presented different trends for spring and autumn and for the taxonomic and biological trait approaches. In spring, while the genus richness and all taxonomic diversity indexes, including the biological index IBMWP, increased with the distance from the dams, the functional metrics only showed a slight increase for Simpson and Shannon trait diversity. These different trends showed that an increase in the taxonomic values with increasing distance did not generate an increase in function for this season. The observed trend of higher taxonomic richness and diversity with increasing distance from the dams in spring can be due to the absence or low abundances in the first reaches of those species considered sensitive to metal and organochlorine pollution such as *E. virgo* (De Haas et al., 2002), which are only present in spring due to its univoltine life cycle. Thus, we should consider that sensitive species can create a spatial pattern only in that period which is not reflected in most of the metrics of the biological traits. On the other hand, the observed decreasing gradient of chlorophyll and $N\text{-}NO_2$ concentration with increasing distance from the dams could have an indirect effect on macroinvertebrate composition. In autumn, the picture was different since the community metrics presented higher values and changes along the spatial gradient were not so marked. It might be due to the mentioned homogenization of the community after the flow stability period and, as explained above, to the absence of species that can be more affected by the pollution and dam impacts in the area. Moreover, the disturbance due to pollutant loading can be higher in spring in the lower Ebro, related with the high flows released in April that might had provoked a remobilization of sediments from the dam and affect at a major level the reaches close to the source. Therefore, a higher discharge event might be combined with a higher pollution release from the sediment dump in spring.

4.3 Relationship of taxonomic and functional community metrics

The explicit measures of functional diversity compared to those based on taxonomic-structure can reveal alternative results since the functional differences in a community are considered (e.g. Bady et al., 2005; Bonada et al., 2007a). Moreover, the incorporation of several metrics as the widely applied richness or Simpson's and Shannon's diversity (emphasizing the evenness and richness component, respectively) to Rao's diversity (based on dissimilarities) can contribute to a better interpretation of functional diversity (Díaz & Cabido, 2001; Bêche & Statzner, 2009). Within all metrics used, functional Rao diversity appeared to be the best metric to distinguish the temporal variability according to the different hydrological situations. Moreover, it showed that despite the slight differences observed for the other metrics, the functional dissimilarities were not so important along the impact gradient. However, since functional metrics detect the increase or decrease in function but not which traits vary along the gradient, the approach presented by Péru & Dolédec (2010) combining both functional metrics based with all traits and with each individual trait should be a considered in future bioassessment studies.

In agreement with Bêche & Resh (2007), a high correlation between several functional metrics was found in our study (Table 6). Although our analysis is at a local scale, the trait Rao's diversity was correlated with all the other functional measures of diversity, indicating the summarizing power of this index (Champely & Chessel, 2002). Moreover, the Rao's diversity is considered one of the most appropriate indexes for measuring functional diversity when using multiple traits (Botta-Dukat, 2005). On the other hand, the relationship between the taxonomic and functional metrics was reflected by the trait Simpson's diversity, which was significantly correlated with all taxonomic measures, followed by trait Shannon's diversity which was not only significantly related with genus richness. Comparable results were also obtained by Bêche & Resh (2007) and Statzner et al. (2007) regarding the correlation of the trait Simpson's diversity with taxonomic metrics. Recently, Péru & Dolédec (2010) also obtained a positive relationship between trait Rao's index and taxonomic richness and Simpson's index. According to the framework presented by Poff (1997a) and Statzner et al. (2004), the correlation of taxonomy and traits in running waters occurs because traits might be filtered at multiple spatial scales by environmental factors which determine the local

trait composition, thus being under an important influence of the abiotic constraints (Bêche & Statzner, 2009). However, the significant positive relationship obtained by

Table 6 Pearson correlation analysis of community metrics based on taxonomy and biological traits considering all data and both seasons. The level of significance is indicated with * (P<0.05), ** (P<0.01).

		Taxonomy				**Biological traits**			
		Richness	**Simpson**	**Shannon**	**Rao**	**Richness**	**Simpson**	**Shannon**	**Rao**
Taxonomy	**Richness**	.							
	Simpson	.70*	.						
	Shannon	.80**	.98**	.					
	Rao	0.38	0.47	0.51	.				
Biological traits	**Richness**	0.09	0.1	0.16	0.31	.			
	Simpson	.70*	.73*	.82**	.66*	0.36	.		
	Shannon	0.52	.72*	.78**	.68*	0.56	.93**	.	
	Rao	0.19	0.57	0.58	0.57	.72*	.64*	.83**	.

Bêche & Resh (2007) between trait Rao's diversity and taxa richness and between trait and taxa richness was not observed in our study. A possible explanation for not detecting any relationship between trait richness and taxon richness is the scale of the study. Because this two metrics do not follow a linear relationship when analyzing large scale datasets with higher species richness (see Statzner et al., 2007 and Bêche and Statzner, 2009), it is difficult to detect any relationship if samples correspond to those having relatively low taxonomic richness but high trait richness (Fig. 14). Thus, trait richness can be greater than expected since the genus-poor sites are not necessarily always the poorest in trait categories (Statzner et al., 2007).

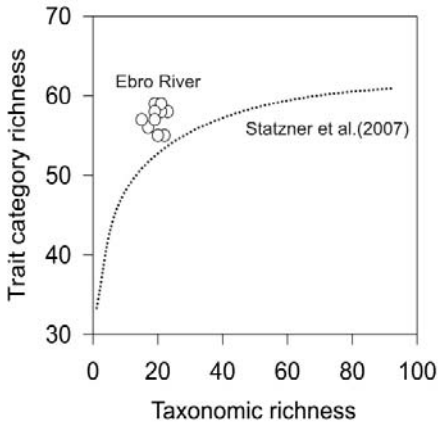


Fig. 14 Contextualization of the lower Ebro samples into the relationship between taxonomic richness and trait category richness obtained by Statzner at al. (2007) for European rivers.

To our knowledge, only a few authors have discussed the correlation of explicit taxonomic diversity measures with functional ones in freshwaters (e.g. Bêche & Resh, 2007; Statzner et al., 2007; Bêche & Statzner, 2009; Péru & Dolédec, 2010). Thus, despite the local scale of this study, the extension of the classical taxonomic approach by the use of functional diversity metrics provided contrasting results to be addressed in human impacted rivers.

4.4 General remarks

In summary, our results reflected a higher relevance of the temporal variability compared to the longitudinal gradient mainly for the macroinvertebrate functional composition in the lower Ebro River, since the taxonomy showed a lower response to temporal changes. This temporal variability seemed to be related with the different hydrological conditions, which changed according to the dam operations. In wet years, the dam operations can reflect the natural flow events but decreased in intensity. Therefore, since our study was carried out in a wet year (2007), the spring floods together with the presence of point source pollution created clearer temporal and spatial patterns than in dry years when the river flow remains more constant.

For the studied period, the autumn floods which occurred in historically natural conditions did not exist in the hydrograph and constant flows prevailed. Although this stable habitat conditions after the high flow in spring created a wider trait category spectrum, these results are disconnected from what would be expected under a situation closer to natural events. The adapted life history traits of native species to predictable natural flow events (Lytle & Poff, 2004) will be desynchronized of the environmental constraints. For instance, the absence of native freshwater mussels along all reaches and both seasons was an indicative of their decline since their complex life cycles depending on fish might be highly affected. Moreover, the higher water conductivity and nutrient load in autumn due to a combination of land uses and lower flow can be a key factor determining the dynamics of the macroinvertebrate community. Although the different hydrological situations created a temporal heterogeneity in trait composition, Statzner & Bêche (2010) pointed out that it can be difficult to distinguish those traits responding to discharge variation without direct physical measurements (e.g. velocity, shear stress). For this reason, further research on the trait response to hydraulic conditions is needed.

When assessing the impact gradient from the dams, the trait response was related mostly to feeding habits, locomotion and reproduction. However, the strong temporal effect together with the phylogenetic link of the sensitivity of species to pollution could have blurred our results. Moreover, in the context of a sediment dump, the combined effect of a high water discharge with higher pollution exposures could occur since remobilization of sediments is also higher under this situation.

In agreement with the multivariate analysis, functional metrics distinguished better the temporal changes than the taxonomic metrics. Functional Rao diversity clearly showed the higher trait dissimilarity after habitat stability, since many habitats were colonized and different strategies cohabited. On the other hand, the taxonomic metrics, including the biotic index, followed better the impact gradient, but only in spring, due to the presence of relatively pollutant sensitive species such as *E. virgo* and a higher pollutant exposure due to the sediment remobilization after a higher discharge. Functional metrics only detected slight changes in the community along the pollution gradient which indicates that a mechanistic understanding of selected trait modalities would be necessary for a better performance of traits as indicators of biological impairment (Statzner & Dolédec, 2008).

Because our study was limited to wadeable areas and they are those more affected by discharge variation, the temporal and longitudinal patterns on the main deep channel could differ and further research in these areas in needed. However, since wadeable areas are those more frequently sampled for bioassessment purposes in gravel-bed rivers where methods as dredges cannot be applied, the present study provides a good approach in the study of the macroinvertebrate community in the lower Ebro River. On the other hand, due to the dominance of Chironomidae and Oligochaeta in this study, a higher taxonomic resolution for these groups would be necessary in the future for a better characterization of the macroinvertebrate distribution. Moreover, a study considering a larger spatial scale in the Ebro River, including the highly diverse mid-Ebro floodplain (see Gallardo et al., 2008, 2009, 2009b, 2009c), would improve the understanding of the community changes downstream of the main impact sources (dams and sediment dump).

5. Acknowledgements

This study was funded by the Government of Catalonia (Agència Catalana de l'Aigua), the European Social Fund and the Government of Spain (Ministerio de Educación y Ciencia, research project CGL2006-01487, Plan Nacional I+D+I; Ministerio de Medioambiente y Medio Rural y Marino). We thank the Ebro Water Authority (Confederación Hidrográfica del Ebro) for the data on water discharge. We are very grateful to Sylvain Dolédec (Traits database), and all the sampling team of the Aquatic Ecosystems-IRTA. Thanks to Manuel Graça, Maria Joao Feijo for their useful comments.

6. References

Alba-Tercedor, J. & A. Sánchez-Ortega, 1988. Un método rápido y simple para evaluar la calidad biológica de las aguas corrientes basado en el de Hellawell (1978). Limnetica 4: 51–56.

Alba-Tercedor, J., P. Jáimez-Cuéllar, M. Alvarez, J. Avilés, N. Bonada, J. Casas, A. Mellado, M. Ortega, I. Pardo, N. Prat, M. Rieradevall, S. Robles, C. E. Sáinz-Cantero, A. Sánchez-Ortega, M. L. Suárez, M. Toro, M. R. Vidal- Albarca, S. Vivas & C. Zamora-Muñoz, 2004. Caracterización del estado ecológico de los ríos mediterráneos ibéricos mediante el índice IBMWP (antes BMWP'). Limnetica (2002) 21(3–4): 175–185.

Altaba, C. R. 1990. The last known population of the fresh-water mussel margaritifera-auricularia (bivalvia, unionoida) - a conservation priority. Biological Conservation 52:271-286.

Altaba, C. R. 1997. At extinction's edge: Margaritifera auricularia (bivalvia: Unionoida). Butlleti de la Institucio Catalana d'Historia Natural 65:137-147.

Araujo, R. & M. A. Ramos. 2000. Status and conservation of the giant European freshwater pearl mussel (*Margaritifera auricularia*) (Spengler, 1793) (Bivalvia : Unionoidea). Biological Conservation 96:233-239.

Archaimbault, V., P. Usseglio-Polatera, J. Garric, J. G. Wasson & M. Babut. 2010. Assessing pollution of toxic sediment in streams using bio-ecological traits of benthic macroinvertebrates. Freshwater Biology 55:1430-1446.

Bady, P., S. Dolédec, C. Fesl, S. Gayraud, M. Bacchi & F. Scholl. 2005. Use of invertebrate traits for the biomonitoring of European large rivers: The effects of sampling effort on genus richness and functional diversity. Freshwater Biology 50:159-173.

Barata, C., M. C. Fabregat, J. Cotín, D. Huertas, M. Solé, L. Quirós, C. Sanpera, L. Jover, X. Ruiz, J. O. Grimalt & B. Piña. 2010. Blood biomarkers and contaminant

levels in feathers and eggs to assess environmental hazards in heron nestlings from impacted sites in Ebro basin (NE Spain). Environmental Pollution 158:704-710.

Batalla, R. J. & D. Vericat. 2009. Hydrological and sediment transport dynamics of flushing flows: Implications for management in large Mediterranean rivers. River Research and Applications 25:297-314.

Bêche, L. A., E. P. McElravy & V. H. Resh. 2006. Long-term seasonal variation in the biological traits of benthic-macroinvertebrates in two Mediterranean-climate streams in california, USA. Freshwater Biology 51:56-75.

Bêche, L. A. & V. H. Resh. 2007. Biological traits of benthic macroinvertebrates in california Mediterranean-climate streams: Long-term annual variability and trait diversity patterns. Fundamental and Applied Limnology 169:1-23.

Bêche, L. A. & B. Statzner. 2009. Richness gradients of stream invertebrates across the USA: Taxonomy- and trait-based approaches. Biodiversity and Conservation 18:3909-3930.

Biggs, B. J. F. 2000. Eutrophication of streams and rivers: Dissolved nutrient-chlorophyll relationships for benthic algae. Journal of the North American Benthological Society 19:17-31.

Bonada, N., S. Dolédec & B. Statzner. 2007a. Taxonomic and biological trait differences of stream macroinvertebrate communities between Mediterranean and temperate regions: Implications for future climatic scenarios. Global Change Biology 13:1658-1671.

Bonada, N., N. Prat, V. H. Resh & B. Statzner. 2006. Developments in aquatic insect biomonitoring: A comparative analysis of recent approaches. Annual Review of Entomology 51:495-523.

Bonada, N., M. Rieradevall & N. Prat. 2007b. Macroinvertebrate community structure and biological traits related to flow permanence in a Mediterranean river network. Hydrobiologia 589:91-106.

Bosch, C., A. Olivares, M. Faria, J. M. Navas, I. del Olmo, J. O. Grimalt, B. Piña & C. Barata. 2009. Identification of water soluble and particle bound compounds causing sublethal toxic effects. A field study on sediments affected by a chlor-alkali industry. Aquatic Toxicology 94:16-27.

Botta-Dukat, Z. 2005. Rao's quadratic entropy as a measure of functional diversity based on multiple traits. Journal of Vegetation Science 16:533-540.

Buchwalter, D. B., D. J. Cain, C. A. Martin, L. Xie, S. N. Luoma & T. Garland, Jr. 2008. Aquatic insect ecophysiological traits reveal phylogenetically based differences in dissolved cadmium susceptibility. Proceedings of the National Academy of Sciences of the United States of America 105:8321-8326.

Buffagni, A., S. Erba, M. Cazzola, J. Murray-Bligh, H. Soszka & P. Genoni. 2006. The star common metrics approach to the wfd intercalibration process: Full application for small, lowland rivers in three european countries. Hydrobiologia 566:379-399.

Carol, J., L. Benejam, J. Benito & E. García-Berthou. 2009. Growth and diet of European catfish (*Silurus glanis*) in early and late invasion stages. Fundamental and Applied Limnology 174:317-328.

Champely, S. & D. Chessel. 2002. Measuring biological diversity using euclidean metrics. Environmental and Ecological Statistics 9:167-177.

Charvet, S., B. Statzner, P. Usseglio-Polatera & B. Dumont. 2000. Traits of benthic macroinvertebrates in semi-natural french streams: An initial application to biomonitoring in europe. Freshwater Biology 43:277-296.

Chevenet, F., S. Dolédec & D. Chessel. 1994. A fuzzy coding approach for the analysis of long-term ecological data Freshwater Biology 31:295-309.

Cid, N., C. Ibáñez, A. Palanques & N. Prat. 2010. Patterns of metal bioaccumulation in two filter-feeding macroinvertebrates: Exposure distribution, inter-species differences and variability across developmental stages. Science of the Total Environment 408:2795-2806.

Clements, W. H. 1999. Metal tolerance and predator-prey interactions in benthic macroinvertebrate stream communities. Ecological Applications 9:1073-1084.

De Haas, E. M., B. Reuvers, C. T. A. Moermond, A. A. Koelmans & M. H. S. Kraak. 2002. Responses of benthic invertebrates to combined toxicant and food input in floodplain lake sediments. Environmental Toxicology and Chemistry 21:2165-2171.

De Lange, H. J., J. De Jonge, P. J. Den Besten, J. Oosterbaan & E. Peeters. 2004. Sediment pollution and predation affect structure and production of benthic macroinvertebrate communities in the rhine-meuse delta, the netherlands. Journal of the North American Benthological Society 23:557-579.

Díaz, S. & M. Cabido. 2001. Vive la difference: Plant functional diversity matters to ecosystem processes. Trends in Ecology & Evolution 16:646-655.

Dolédec, S. & B. Statzner. 2008. Invertebrate traits for the biomonitoring of large European rivers: An assessment of specific types of human impact. Freshwater Biology 53:617-634.

Dolédec, S., B. Statzner & M. Bournard. 1999. Species traits for future biomonitoring across ecoregions: Patterns along a human-impacted river. Freshwater Biology 42:737-758.

Ducrot, V., P. Usseglio-Polatera, A. R. R. Pery, J. Mouthon, M. Lafont, M. C. Roger, J. Garric & J. F. Ferard. 2005. Using aquatic macroinvertebrate species traits to build test batteries for sediment toxicity assessment: Accounting for the diversity of potential biological responses to toxicants. Environmental Toxicology and Chemistry 24:2306-2315.

European Commission. 2000. Directive 2000/60/EC of the European Parliament and of the Council. Establishing a framework for Community action in the field of water policy, Brussels, Belgium.

Gallardo, B., M. García, A. Cabezas, E. González, M. González, C. Ciancarelli & F. A. Comin. 2008. Macroinvertebrate patterns along environmental gradients and hydrological connectivity within a regulated river-floodplain. Aquatic Sciences 70:248-258.

Gallardo, B., S. Gascón, A. Cabezas, M. González, M. García & F. A. Comín. 2009a. Relationship between invertebrate traits and lateral environmental gradients in a Mediterranean river-floodplain. Fundamental and Applied Limnology 173:281-292.

Gallardo, B., S. Gascón, M. García & F. A. Comín. 2009b. Testing the response of macroinvertebrate functional structure and biodiversity to flooding and confinement. Journal of Limnology 68:315-326.

Gallardo, B., S. Gascón, M. González-Sanchis, A. Cabezas & F. A. Comín. 2009c. Modelling the response of floodplain aquatic assemblages across the lateral hydrological connectivity gradient. Marine and Freshwater Research 60:924-935.

Gasith, A. & V. H. Resh. 1999. Streams in Mediterranean climate regions: Abiotic influences and biotic responses to predictable seasonal events. Annual Review of Ecology and Systematics 30:51-81.

Gayraud, S., B. Statzner, P. Bady, A. Haybachp, F. Scholl, P. Usseglio-Polatera & M. Bacchi. 2003. Invertebrate traits for the biomonitoring of large European rivers: An initial assessment of alternative metrics. Freshwater Biology 48:2045-2064.

Gjerlov, C., A. G. Hildrew & J. I. Jones. 2003. Mobility of stream invertebrates in relation to disturbance and refugia: A test of habitat templet theory. Journal of the North American Benthological Society 22:207-223.

Graffhoff, K., K.Kremling & M.Ehrhardt. 1999. Methods of seawater analysis. Wiley-VHC, Weinheim. 577p.

Grimalt, J.O., 2006. http://mediambient.gencat.cat/cat/ciutadans/informacioambiental/Flix/inici.jsp.

Guillén, J. & A. Palanques. 1992. Sediment dynamics and hydrodynamics in the lower course of a river highly regulated by dams - the Ebro river. Sedimentology 39:567-579.

Henle, K., M. Scholz, F. Dziock, S. Stab & F. Foeckler. 2006. Bioindication and functional response in floodplain systems: Where to from here? International Review of Hydrobiology 91:380-387.

Ibáñez C, Rodrígues-Capítulo A, & Prat N. 1995. The combined impacts of river regulation and eutrophication on the dynamics of the salt wedge and the ecology of the lower Ebro River. In: Harper, D. & Ferguson, A. (eds.) The ecological basis for river management, p 105-114. John Wiley & Sons, Chichester.

Ibáñez, C., D. Pont & N. Prat. 1997. Characterization of the ebre and rhone estuaries: A basis for defining and classifying salt-wedge estuaries. Limnology and Oceanography 42:89-101.

Ibáñez, C., N. Prat, C. Duran, M. Pardos, A. Munné, R. Andreu, N. Caiola, N. Cid, H. Hampel, R. Sánchez & R. Trobajo. 2008. Changes in dissolved nutrients in the lower Ebro river: Causes and consequences. Limnetica 27:131-142.

Ihaka, R., & R. Gentleman. 1996. R: a language for data analysis and graphics. Journal of Computational and Graphical Statistics 5: 299–314.

Jiménez-Muer, P. 2001. Localization and evaluation of a new biological invasion: the zebra mussel (*Dreissena polymorpha*) in the Ebro. www.agua-debate.org/htm/mcebra/docmc/mma_freixe.pdf2001.

Klok, C. & M. H. S. Kraak. 2008. Living in highly dynamic polluted river floodplains, do contaminants contribute to population and community effects? Science of the Total Environment 406:455-461.

Lacorte, S., D. Raldúa, E. Martínez, A. Navarro, S. Díez, J. M. Bayona & D. Barceló. 2006. Pilot survey of a broad range of priority pollutants in sediment and fish from the Ebro river basin (ne Spain). Environmental Pollution 140:471-482.

Lecerf, A., P. Usseglio-Polatera, J. Y. Charcosset, D. Lambrigot, B. Bracht & E. Chauvet. 2006. Assessment of functional integrity of eutrophic streams using litter breakdown and benthic macroinvertebrates. Archiv Fur Hydrobiologie 165:105-126.

Lytle, D. A. & N. L. Poff. 2004. Adaptation to natural flow regimes. Trends in Ecology & Evolution 19:94-100.

Mackay, R. J. 1992. Colonization by lotic macroinvertebrates - a review of processes and patterns. Canadian Journal of Fisheries and Aquatic Sciences 49:617-628.

Mañosa, S., R. Mateo & R. Guitart. 2001. A review of the effects of agricultural and industrial contamination on the Ebro delta biota and wildlife. Environmental Monitoring and Assessment 71:187-205.

Mellado- Díaz, A., M. L. Suárez Alonso & M. R. Vidal-Abarca Gutiérrez. 2008. Biological traits of stream macroinvertebrates from a semi-arid catchment: Patterns along complex environmental gradients. Freshwater Biology 53:1-21.

Mouillot, D., S. Spatharis, S. Reizopoulou, T. Laugier, L. Sabetta, A. Basset & T. D. Chi. 2006. Alternatives to taxonomic-based approaches to assess changes in transitional water communities. Aquatic Conservation-Marine and Freshwater Ecosystems 16:469-482.

Munné, A. & N. Prat. 2009. Use of macroinvertebrate-based multimetric indices for water quality evaluation in Spanish Mediterranean rivers: An intercalibration approach with the IBMWP index. Hydrobiologia 628:203-225.

Muñoz, I. & N. Prat. 1994. Macroinvertebrate community in the lower Ebro river (ne Spain) Hydrobiologia 286:65-78.

Navarro, E., M. Bacardit, L. Caputo, T. Palau & J. Armengol. 2006. Limnological characterization and flow patterns of a three-coupled reservoir system and their influence on *Dreissena polymorpha* populations and settlement during the stratification period. Lake and Reservoir Management 22:293-302.

Navarro, A., L. Quirós, M. Casado, M. Faria, L. Carrasco, L. Benejam, J. Benito, S. Díez, D. Raldúa, C. Barata, J. M. Bayona & B. Piña. 2009. Physiological responses to mercury in feral carp populations inhabiting the low Ebro River (NE Spain), a historically contaminated site. Aquatic Toxicology 93:150-157.

Oksanen, J., F.G. Blanchet, , R. Kindt, P. Legendre, R.G. O'Hara, G.L. Simpson, P. Solymos, H. Stevens & H. Wagner. 2010. Vegan: Community Ecology Package. R package version 1.17-0. http://CRAN.R-project.org/package=vegan

Péru, N. & S. Dolédec. 2010. From compositional to functional biodiversity metrics in bbioassessment: A case study using macroinvertebarte communities. Ecological Indicators 10: 1025-1036.

Petts G.E., H. Möller & A.L. Roux (Eds). 1993. Historical change of alluvial rivers: Western Europe. John Wiley & Sons, New York.

Poff, N. L. 1997a. Landscape filters and species traits: Towards mechanistic understanding and prediction in stream ecology. Journal of the North American Benthological Society 16:391-409.

Poff, N. L., J. D. Allan, M. B. Bain, J. R. Karr, K. L. Prestegaard, B. D. Richter, R. E. Sparks & J. C. Stromberg. 1997b. The natural flow regime. Bioscience 47:769-784.

Poff, N. L., J. D. Olden, N. K. M. Vieira, D. S. Finn, M. P. Simmons & B. C. Kondratieff. 2006. Functional trait niches of North American lotic insects: Traits-based ecological applications in light of phylogenetic relationships. Journal of the North American Benthological Society 25:730-755.

Poff N.L., J.D. Olden, D.M. Merritt & D.M. Pepin. 2007. Homogenization of regional river dynamics by dams and global biodiversity implications. Proceedings of the National Academy of Sciences of the United States of America 104: 5732–5737.

Pollard, A. I. & L. Yuan. 2006. Community response patterns: Evaluating benthic invertebrate composition in metal-polluted streams. Ecological Applications 16:645-655.

Prats, J., R. Val, J. Armengol & J. Dolz. 2010. Temporal variability in the thermal regime of the lower ebro river (spain) and alteration due to anthropogenic factors. Journal of Hydrology 387:105-118.

Puntí, T., M. Rieradevall & N. Prat. 2009. Environmental factors, spatial variation, and specific requirements of chironomidae in Mediterranean reference streams. Journal of the North American Benthological Society 28:247-265.

Ramos, L., M. A. Fernández, M. J. González & L. M. Hernández. 1999. Heavy metal pollution in water, sediments, and earthworms from the Ebro river, Spain. Bulletin of Environmental Contamination and Toxicology 63:305-311.

Resh, V. H., A. G. Hildrew, B. Statzner & C. R. Townsend. 1994. Theoretical habitat templets, species traits, and species richness - a synthesis of long-term ecological research on the upper Rhone River in the context of concurrently developed ecological theory. Freshwater Biology 31:539-554.

Rosenberg, D. M. & V. H. Resh. 1993. Freshwater biomonitoring and benthic macroinvertebrates. Chapmann and Hall, New York. 488p.

Sabater, S., J. Artigas, C. Duran, M. Pardos, A. M. Romaní, E. Tornés & I. Ylla. 2008. Longitudinal development of chlorophyll and phytoplankton assemblages in a regulated large river (the Ebro River). Science of the Total Environment 404:196-206.

Solà, C., M. Burgos, A. Plazuelo, J. Toja, M. Plans & N. Prat. 2004. Heavy metal bioaccumulation and macroinvertebrate community changes in a Mediterranean stream affected by acid mine drainage and an accidental spill (Guadiamar River, SW Spain). Science of the Total Environment: 333:109–26.

Southwood, T. R. E. 1988. Tactics, strategies and templets. Oikos 52:3-18.

Statzner, B., P. Bady, S. Dolédec & F. Scholl. 2005. Invertebrate traits for the biomonitoring of large European rivers: An initial assessment of trait patterns in least impacted river reaches. Freshwater Biology 50:2136-2161.

Statzner, B. & L. A. Bêche. 2010. Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? Freshwater Biology 55:80-119.

Statzner, B., B. Bis, S. Dolédec & P. Usseglio-Polatera. 2001. Perspectives for biomonitoring at large spatial scales: A unified measure for the functional composition on invertebrate communities in European running waters. Basic and Applied Ecology 2:73-85.

Statzner, B., N. Bonada & S. Dolédec. 2007. Conservation of taxonomic and biological trait diversity of European stream macroinvertebrate communities: A case for a collective public database. Biodiversity and Conservation 16:3609-3632.

Statzner, B., S. Dolédec & B. Hugueny. 2004. Biological trait composition of European stream invertebrate communities: Assessing the effects of various trait filter types. Ecography 27:470-488.

Statzner, B., A. G. Hildrew & V. H. Resh. 2001. Species traits and environmental, constraints: Entomological research and the history of ecological theory. Annual Review of Entomology 46:291-316.

Statzner, B., K. Hoppenhaus, M. F. Arens & P. Richoux. 1997. Reproductive traits, habitat use and templet theory: A synthesis of world-wide data on aquatic insects. Freshwater Biology 38:109-135.

Steinman, A.D. & G. A. Lamberti. 1996. Biomass and pigments of benthic algae. P. 297. In "Methods in Stream Ecology". Hauer, F.R. and G.A. Lamberti (eds). Academic Press, San Diego, CA.

Strayer, D. L., M. L. Pace, N. F. Caraco, J. J. Cole & S. E. G. Findlay. 2008. Hydrology and grazing jointly control a large-river food web. Ecology 89:12-18.

Suárez-Serrano, A., C. Alcaraz, C. Ibáñez, R. Trobajo & C. Barata. 2010. *Procambarus clarkii* as a bioindicator of heavy metal pollution sources in the lower Ebro River and delta. Ecotoxicology and Environmental Safety 73:280-286.

Suren, A. M. & I. G. Jowett. 2006. Effects of floods versus low flows on invertebrates in a New Zealand gravel-bed river. Freshwater Biology 51:2207-2227.

Tachet, H., P. Ricoux, M. Bournaud & P. Usseglio- Polatera 2000. Invertebrés d'eau douce: Systématique, biologie, écologie. Paris, CNRS Editions. Pages pp.

Terrado, M., D. Barceló & R. Tauler. 2006. Identification and distribution of contamination sources in the Ebro basin by chemometrics modelling coupled to geographical information systems. Talanta 70:691-704.

Thioulouse, J., D. Chessel, S. Dolédec & J. M. Olivier. 1997. Ade-4: A multivariate analysis and graphical display software. Statistics and Computing 7:75-83.

Tomanova, S., N. Moya & T. Oberdorff. 2008. Using macroinvertebrate biological traits for assessing biotic integrity of neotropical streams. River Research and Applications 24:1230-1239.

Townsend, C. R. 1989. The patch dynamics concept of stream community ecology. Journal of the North American Benthological Society 8:36-50.

Townsend, C. R., S. Dolédec & M. R. Scarsbrook. 1997. Species traits in relation to temporal and spatial heterogeneity in streams: A test of habitat templet theory. Freshwater Biology 37:367-387.

Townsend, C. R. & A. G. Hildrew. 1994. Species traits in relation to a habitat templet for river systems Freshwater Biology 31:265-275.

Usseglio-Polatera, P. & J. N. Beisel. 2002. Longitudinal changes in macroinvertebrate assemblages in the Meuse River: Anthropogenic effects versus natural change. River Research and Applications 18:197-211.

Usseglio-Polatera, P. 1994. Theoretical habitat templets, species traits, and species richness - aquatic insects in the upper rhone river and its floodplain. Freshwater Biology 31:417-437.

Vericat, D. & R. J. Batalla. 2006. Sediment transport in a large impounded river: The lower Ebro, NE Iberian Peninsula. Geomorphology 79:72-92.

Williams, D. D. 1996. Environmental constraints in temporary fresh waters and their consequences for the insect fauna. Journal of the North American Benthological Society 15:634-650.

SUPPORTING INFORMATION

SI1. Summary of the mean vaules (±SD) of physical and chemical variables measured at the five studied reaches for each season. For every variable n=3.

		E01		E02		E03		E04		E05	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Tª (ºC)	Spring	19.4	0.1	19.2	0.1	21.1	0.0	20.4	0.1	18.5	0.1
	Autumn	21.5	0.0	22.6	0.0	22.2	0.1	22.6	0.0	21.5	0.0
pH	Spring	8.0	0.0	8.0	0.1	8.1	0.0	8.1	0.0	8.1	0.0
	Autumn	8.3	0.0	8.3	0.0	8.2	0.0	8.3	0.0	8.3	0.0
Cond (µs/cm)	Spring	709	2	704	1	683	1	674	1	660	2
	Autumn	1456	3	1474	1	1463	2	1474	1	1456	3
Salinity (ppt)	Spring	0.39	0.00	0.39	0.00	0.36	0.00	0.36	0.00	0.37	0.00
	Autumn	0.79	0.00	0.78	0.00	0.78	0.00	0.78	0.00	0.79	0.00
DO (mg/l)	Spring	9.637	0.015	9.093	0.450	9.583	0.031	8.800	0.053	9.077	0.310
	Autumn	7.847	0.099	9.077	0.110	7.880	0.130	9.077	0.110	7.847	0.099
DO (%)	Spring	105.0	0.1	98.6	5.2	107.8	0.4	97.7	0.8	97.1	3.6
	Autumn	89.3	1.1	105.4	1.3	90.9	1.5	105.4	1.3	89.3	1.1
SPM (mg/l)	Spring	3.35	0.48	2.95	0.70	3.57	0.10	2.97	0.64	2.00	0.30
	Autumn	2.34	0.08	2.77	0.17	2.77	0.10	2.75	0.21	3.64	0.25
OM (mg/l)	Spring	0.63	0.23	0.77	0.13	1.27	0.16	1.05	0.35	0.73	0.18
	Autumn	0.88	0.09	0.89	0.09	0.84	0.06	0.68	0.09	0.97	0.04
OM (%)	Spring	19.9	10.6	26.5	3.6	35.6	5.6	34.9	6.0	36.4	4.0
	Autumn	37.3	2.5	32.2	2.5	30.3	1.7	24.8	1.3	26.6	2.2
Chl a (µg/l)	Spring	0.605	0.339	0.446	0.234	0.578	0.328	1.085	1.455	0.810	0.427
	Autumn	1.066	0.205	0.587	0.163	0.876	0.200	1.445	0.016	2.033	0.195
Phaeo (µg/l)	Spring	0.675	0.336	0.713	0.329	0.783	0.514	0.275	0.073	0.953	0.449
	Autumn	0.859	0.182	0.860	0.312	1.361	0.317	1.493	0.198	2.130	0.099
P-PO4 (mg/l)	Spring	0.016	0.002	0.017	0.004	0.016	0.003	0.015	0.003	0.011	0.001
	Autumn	0.030	0.006	0.042	0.003	0.041	0.001	0.045	0.007	0.045	0.006
TDP (mg/l)	Spring	0.040	0.001	0.039	0.002	0.033	0.001	0.036	0.002	0.034	0.003
	Autumn	0.071	0.006	0.069	0.004	0.065	0.006	0.069	0.007	0.075	0.005
$N\text{-}NH_4$ (mg/l)	Spring	0.016	0.003	0.029	0.003	0.024	0.004	0.033	0.001	0.068	0.003
	Autumn	0.060	0.025	0.080	0.018	0.047	0.044	0.070	0.035	0.036	0.027
$N\text{-}NO_2$ (mg/l)	Spring	0.023	0.001	0.017	0.001	0.019	0.002	0.026	0.006	0.048	0.003
	Autumn	0.003	0.000	0.009	0.000	0.013	0.001	0.022	0.002	0.056	0.005
$N\text{-}NO_3$ (mg/l)	Spring	1.932	0.113	1.915	0.074	1.236	0.087	1.472	0.288	1.409	0.105
	Autumn	1.847	0.108	2.371	0.281	2.437	0.070	2.399	0.061	2.434	0.021
TDN (mg/l)	Spring	2.140	0.006	2.161	0.058	1.623	0.160	1.632	0.204	1.815	0.050
	Autumn	2.649	0.047	2.816	0.050	2.721	0.154	2.794	0.013	2.851	0.015
Si (mg/l)	Spring	2.250	0.036	2.266	0.013	2.168	0.038	2.113	0.052	2.010	0.048
	Autumn	1.760	0.392	1.309	0.384	1.726	0.491	1.847	0.102	1.462	0.254

SI 2. Abundance of the macroinvertebrate taxa used for the analysis.

	Spring					Autumn				
Taxon	**E01**	**E02**	**E03**	**E04**	**E05**	**E01**	**E02**	**E03**	**E04**	**E05**
Hydra sp.	0.0	13.0	4.0	14.0	0.8	0.0	3.0	1.0	3.0	52.2
Dugesia sícula Lepori	0.6	1.5	2.0	1.0	0.2	2.3	18.5	16.0	3.3	53.2
Prostoma graecense (Böhmig)	0.0	1.3	0.4	0.0	0.0	1.3	7.8	21.0	10.0	233.0
Mermithidae	0.0	1.0	0.8	0.0	0.0	0.5	0.0	2.6	0.0	0.0
Oligochaeta	357.6	548.5	330.8	168.2	97.8	11.5	20.0	170.8	70.0	535.8
Helobdella stagnalis (Linnaeus)	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.2	0.3	0.0
Corbicula fluminea (O.F. Müller)	36.6	13.0	26.2	25.8	2.4	52.0	126.3	100.2	39.3	2.6
Dreissena polymorpha (Pallas)	0.2	0.0	0.4	0.6	0.6	0.0	0.0	0.0	0.0	1.8
Ferrissia (Pettancylus) clessiniana (Jickelli)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.8	0.8
Melanopsis tricarinata dufourii (Férrussac)	0.6	0.0	0.0	0.0	0.0	1.5	0.0	0.0	0.0	0.0
Physella acuta (Draparnaud)	0.0	0.5	2.2	0.0	0.2	0.0	1.8	1.2	0.0	0.0
Radix sp.	0.2	0.0	0.0	0.0	0.0	0.0	0.3	0.0	1.3	0.0
Theodoxus fluviatilis (L.)	0.0	0.3	4.6	0.8	0.0	1.5	3.0	0.0	0.0	0.0
Echinogammarus longisetosus Pinkster	16.8	24.5	42.6	133.0	47.8	0.0	2.5	0.8	0.0	30.8
Proasellus meridianus (Racovitza)	0.6	1.3	1.6	7.0	12.8	0.0	0.3	0.0	0.0	9.0
Baetis sp.	5.2	5.8	0.8	6.4	0.0	7.0	23.8	11.8	1.3	0.2
Caenis luctuosa (Burmeister)	4.8	5.0	8.8	2.2	0.8	1.8	0.3	2.0	9.8	3.8
Choroterpes pictetii (Eaton)	3.8	6.5	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ephoron virgo (Olivier)	14.4	3.5	1.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0
Micronecta sp.	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0	3.3	22.4
Aulonogyrus sp.	0.0	0.5	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Dryops sp.	0.0	0.0	0.2	0.0	0.0	0.8	1.3	0.4	0.0	0.0
Ecnomus tenellus (Rambur)	1.0	0.0	0.2	0.0	0.0	0.3	1.3	0.8	2.3	6.4
Hydropsyche exocellata Dufour	0.2	0.0	0.8	0.8	0.0	0.3	0.3	0.8	1.3	1.4
Hydroptila sp.	0.0	1.3	15.2	0.0	0.4	0.0	1.5	6.8	0.0	10.4
Orthotrichia angustella (McLachlan)	0.2	0.0	0.0	0.0	0.0	0.0	3.0	1.0	0.3	0.0
Psychomyia pusilla (Fabricius)	0.8	0.0	0.0	0.0	0.0	0.3	0.0	0.0	2.8	0.0
Tanypodinae	1.0	0.5	0.0	2.4	3.4	0.3	0.8	0.2	0.5	3.4
Orthocladiinae	186.2	123.8	521.8	456.8	116.8	1.3	9.8	7.6	8.3	22.6
Chironomini	18.2	12.5	0.0	4.8	0.8	0.3	0.0	0.8	0.3	3.2
Tanytarsini	1.2	1.5	0.8	0.2	0.0	0.8	1.0	0.2	0.5	40.8
Ceratopogoninae	0.6	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Simulium erythrocephalum (De Geer)	0.4	0.0	2.4	0.0	0.0	0.5	11.8	4.4	0.0	0.0

SI 3. Relative abundance of traits per sample.

		Spring					Autumn				
Trait	**Category**	**E01**	**E02**	**E03**	**E04**	**E05**	**E01**	**E02**	**E03**	**E04**	**E05**
Maximal size	<0.25 cm	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.039	0.049
	>0.25–0.5 cm	0.106	0.105	0.164	0.085	0.117	0.115	0.166	0.159	0.195	0.150
	>0.5–1 cm	0.338	0.354	0.351	0.363	0.352	0.316	0.317	0.257	0.287	0.310
	>1–2 cm	0.258	0.269	0.211	0.195	0.175	0.240	0.238	0.309	0.233	0.261
	>2–4 cm	0.188	0.187	0.180	0.253	0.256	0.137	0.151	0.139	0.121	0.184
	>4–8 cm	0.110	0.084	0.094	0.103	0.081	0.192	0.128	0.135	0.125	0.047
Life cycle duration	<1 year	0.584	0.491	0.506	0.428	0.418	0.460	0.458	0.426	0.517	0.438
	>1 year	0.416	0.509	0.494	0.572	0.582	0.540	0.542	0.574	0.483	0.562
Potential no. reproductive cycles per year	1	0.358	0.408	0.410	0.353	0.278	0.475	0.520	0.466	0.460	0.457
	>1	0.642	0.592	0.590	0.647	0.722	0.525	0.480	0.534	0.540	0.543
Aquatic stages	Egg	0.297	0.318	0.335	0.289	0.274	0.334	0.343	0.353	0.370	0.305
	Larva	0.395	0.369	0.328	0.360	0.352	0.350	0.316	0.314	0.294	0.321
	Pupa	0.167	0.130	0.135	0.148	0.158	0.073	0.120	0.109	0.101	0.138
	Adult	0.141	0.183	0.201	0.203	0.216	0.243	0.222	0.224	0.235	0.237
Reproduction type	Ovoviviparity	0.209	0.188	0.205	0.310	0.360	0.210	0.163	0.136	0.109	0.182
	Isolated eggs, free	0.168	0.121	0.108	0.075	0.082	0.115	0.077	0.097	0.100	0.050
	Isolated eggs, cemented	0.117	0.109	0.125	0.057	0.033	0.132	0.098	0.061	0.138	0.103
	Clutches, cemented or fixed	0.234	0.289	0.319	0.248	0.207	0.319	0.423	0.479	0.445	0.384
	Clutches, free	0.113	0.094	0.048	0.099	0.108	0.057	0.054	0.043	0.043	0.100
	Clutches, in vegetation	0.000	0.000	0.001	0.000	0.000	0.008	0.006	0.003	0.000	0.000
	Clutches, terrestrial	0.063	0.054	0.076	0.074	0.086	0.046	0.061	0.044	0.032	0.026
	Asexual reproduction	0.095	0.145	0.118	0.136	0.123	0.113	0.119	0.138	0.133	0.155
Dispersal	Aquatic passive	0.525	0.543	0.559	0.632	0.691	0.514	0.448	0.456	0.512	0.484
	Aquatic active	0.200	0.223	0.226	0.192	0.158	0.300	0.338	0.338	0.307	0.333
	Aerial passive	0.137	0.122	0.114	0.085	0.080	0.080	0.107	0.112	0.098	0.113
	Aerial active	0.138	0.113	0.101	0.091	0.071	0.107	0.108	0.094	0.083	0.070
Resistance form	Eggs, statoblasts	0.256	0.219	0.169	0.123	0.122	0.165	0.139	0.161	0.125	0.080
	Cocoons	0.095	0.116	0.102	0.091	0.109	0.162	0.142	0.194	0.162	0.176
	Cells against desiccation	0.027	0.018	0.022	0.025	0.014	0.056	0.038	0.036	0.033	0.007
	Diapause or dormancy	0.069	0.058	0.107	0.071	0.101	0.138	0.115	0.052	0.104	0.122
	None	0.553	0.589	0.600	0.691	0.655	0.480	0.566	0.557	0.575	0.615
Respiration	Tegument	0.555	0.604	0.572	0.535	0.527	0.604	0.626	0.700	0.733	0.701
	Gill	0.441	0.391	0.408	0.462	0.456	0.365	0.323	0.270	0.232	0.256
	Plastron	0.000	0.000	0.002	0.000	0.009	0.011	0.008	0.004	0.017	0.022
	Spiracle (aerial)	0.004	0.005	0.018	0.002	0.009	0.020	0.042	0.026	0.017	0.022
Locomotion and substratum relation	Flier	0.000	0.001	0.002	0.001	0.000	0.008	0.006	0.003	0.000	0.000
	Surface swimmer	0.005	0.026	0.017	0.026	0.008	0.011	0.028	0.020	0.028	0.034
	Full water swimmer	0.236	0.263	0.236	0.253	0.303	0.248	0.261	0.313	0.273	0.321
	Crawler	0.396	0.398	0.428	0.388	0.413	0.417	0.363	0.369	0.359	0.381
	Burrower (epibenthic)	0.210	0.133	0.113	0.113	0.091	0.187	0.129	0.124	0.173	0.059
	Interstitial (endobenthic)	0.075	0.069	0.069	0.095	0.117	0.045	0.051	0.043	0.041	0.058
	Temporarily attached	0.071	0.110	0.130	0.115	0.056	0.080	0.159	0.125	0.112	0.129
	Permanently attached	0.009	0.000	0.006	0.008	0.013	0.004	0.003	0.002	0.013	0.018
Food	Fine sediment and microorganisms	0.042	0.043	0.039	0.035	0.044	0.038	0.022	0.036	0.041	0.039
	Detritus < 1 mm	0.379	0.319	0.291	0.251	0.271	0.259	0.218	0.255	0.220	0.208
	Plant detritus > 1 mm	0.074	0.076	0.088	0.108	0.134	0.085	0.065	0.037	0.058	0.075
	Living microphytes	0.252	0.220	0.241	0.241	0.242	0.248	0.217	0.193	0.228	0.158
	Living macrophytes	0.071	0.080	0.104	0.080	0.085	0.064	0.105	0.099	0.055	0.078
	Dead animal > 1 mm	0.025	0.028	0.029	0.034	0.034	0.026	0.033	0.031	0.060	0.039
	Living microinvertebrates	0.107	0.141	0.133	0.190	0.136	0.110	0.152	0.113	0.174	0.189
	Living macroinvertebrates	0.049	0.092	0.075	0.062	0.054	0.164	0.187	0.235	0.161	0.214
	Vertebrates	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.001	0.002	0.000
Feeding habits	Absorber	0.043	0.067	0.048	0.039	0.052	0.082	0.091	0.137	0.125	0.145
	Deposit feeder	0.290	0.279	0.232	0.207	0.223	0.205	0.126	0.196	0.215	0.180
	Shredder	0.173	0.176	0.186	0.239	0.317	0.110	0.090	0.054	0.085	0.202
	Scraper	0.152	0.150	0.193	0.157	0.127	0.187	0.221	0.151	0.176	0.092
	Filter feeder	0.244	0.182	0.203	0.209	0.136	0.276	0.264	0.234	0.233	0.150
	Piercer (plants or animals)	0.005	0.017	0.040	0.003	0.017	0.023	0.057	0.060	0.036	0.050
	Predator (carver/engulfer/swallower)	0.057	0.081	0.056	0.111	0.090	0.086	0.139	0.115	0.116	0.169
	Parasite	0.034	0.049	0.044	0.036	0.038	0.032	0.012	0.053	0.014	0.014

SI 4. Community metrics.

		Spring					**Autumn**				
		E01	**E02**	**E03**	**E04**	**E05**	**E01**	**E02**	**E03**	**E04**	**E05**
Taxonomy	Richness	22	20	23	17	15	19	21	21	19	19
	Simpson diversity	0.904	0.913	0.911	0.890	0.856	0.894	0.923	0.912	0.917	0.926
	Shannon diversity	2.609	2.672	2.699	2.421	2.198	2.554	2.748	2.664	2.662	2.739
	Rao diversity	0.80	0.59	0.62	0.39	0.36	0.93	0.69	0.59	1.07	0.64
Biological traits	Richness	55	55	58	56	57	59	58	59	58	57
	Simpson diversity	0.969	0.970	0.970	0.968	0.967	0.971	0.971	0.969	0.969	0.970
	Shannon diversity	3.674	3.688	3.698	3.659	3.651	3.717	3.721	3.690	3.704	3.703
	Rao diversity	0.80	0.59	0.62	0.39	0.36	0.93	0.69	0.59	1.07	0.64

Chapter 2

Hydraulic conditions as a key factor for benthic macroinvertebrate assemblages, biological trait response and diversity in a large regulated river.

Cid, N., Ibánez, C., Andreu, R., Collado, R. & Prat, N.

Freshwater Biology **(submitted).**

Hydraulic conditions as a key factor for benthic macroinvertebrate assemblages, biological trait response and diversity in a large regulated river

NÚRIA CID[1†], CARLES IBÁÑEZ[†], ROSA ANDREU[†], RUT COLLADO[‡] & NARCÍS PRAT[*]

* *Department of Ecology, Faculty of Biology, University of Barcelona, Barcelona, Catalonia, Spain.*
† *Aquatic Ecosystems, IRTA, Sant Carles de la Ràpita, Catalonia, Spain.*
‡*Department of Animal and Vegetal Biology and Ecology, University of A Coruña, A Coruña, Galicia, Spain.*

SUMMARY

1. Given that the hydraulic conditions are one of the main factors determining the macroinvertebrate distribution, we characterized the species preferences and their functional structure along a hydraulic gradient in the lower Ebro River (NE Spain) by using the niche separation analysis and the fourth corner method analysis. Quantitative benthic samples were taken simultaneously with the mean water velocity, depth, the percentage cover of macrophytes in the benthic habitat and the interstitial dissolved oxygen. Froude number and Reynolds number were also calculated.

2. Of all the measured parameters, the mean water velocity was the best explanatory variable for the benthic macroinvertebrate distributions and functional structure. All structural and functional metrics were negatively correlated with current velocity while macroinvertebrate densities showed the opposite pattern due to the dominance of a few species in areas with high current velocity.

3. The taxa presenting high hydraulic niche marginality occupied narrower habitat conditions (slow and fast flowing areas) while other species were more tolerant to a wider range of conditions. Chironomidae showed different habitat preferences within the same subfamily, tribe or genus, reflecting the importance of species niche separation.

4. According to the macroinvertebrate distribution, many of the functional characteristics of macroinvertebrates responded to the hydraulic conditions (e.g. feeding, locomotion) while those related with life cycle seemed to reflect adaptations to flow regime events or species interactions.

[1] Correspondence: Núria Cid , Department of Ecology, Faculty of Biology, University of Barcelona, Barcelona, Catalonia, Spain.
E-mail: ncid@ub.edu

5. By linking the macroinvertebrate community metrics, their hydraulic preferences and their biological trait response, we demonstrated that a high taxonomic resolution will improve our understanding of the community response to the hydraulic conditions and therefore increase its potential application in guiding river flow management.

Keywords: hydraulic preferences, biological traits, diversity, macroinvertebrates, Chironomidae, Ebro River

Introduction

The importance of the physical habitat structuring the assemblages of aquatic invertebrates in running waters is widely recognized and the hydraulic conditions are considered one of the main influencing factors (Hynes, 1970; Statzner & Higler 1986; Statzner et al., 1988; Quinn & Hickey, 1994; Hart & Finelli, 1999; Rempel et al., 2000; Mérigoux & Dolédec, 2004). Because of the ecological key role played by invertebrates in stream ecosystems, their study related to the hydraulic habitat has been so far of great interest for stream ecologists in order to understand their distribution, diversity and behavior (Lancaster & Hildrew, 1993; Quinn & Hickey, 1994), as well as ecological processes (Peckarsky et al., 1990; Rader, 1997) and flow adaptations (see Statzner, 2008). In order to characterize the hydraulic conditions, most of these studies included direct measurements near the riverbed, as shear velocity and substrate size composition, and hydraulic parameters as Froude number or Reynolds number (Quinn & Hickey, 1994; Rempel et al., 2000; Mérigoux & Dolédec, 2004). Also the mean water velocity was considered a good explanatory variable of the community composition when assessing mesohabitat hydraulic conditions in areas where substrate composition is relatively homogeneous across the sampled area (Quinn & Hickey, 1994; Syrovatka et al., 2009; Buffagni et al., 2010).

In the context of the River Habitat Templet, the spatial and temporal heterogeneity of the habitat should be reflected in the functional composition of community characteristics as a result of an adaptation to the environmental conditions (Townsend & Hildrew, 1994; Poff et al., 2006). Thus, the adaptation to hydraulic conditions can result in differences of biological traits, as feeding strategies or body size (Rempel et al., 2000; Lamoroux et al., 2004; Mérigoux & Dolédec, 2004; Bonada et al., 2007), being a valuable approach because it gives a good understanding of ecosystem function

(Statzner et al., 2001). Moreover, the trait response to direct hydraulic stressors is crucial when assessing the effect of discharge variability, since it provides a clearer interpretation according to *a priori* hypotheses (Statzner & Bêche, 2010).

On the other hand, the influence of the environmental constraints (in this case, the hydraulic conditions) and interespecific interactions can determine lineage diversification for aquatic insect communities (Múrria, 2010). Thereby, phylogenetically close species can differ in the ecological niche they occupy. If the ecological niche of species at reach scale is mainly determined by hydraulics, there can be species-specific hydraulic preferences within the same family or genus (Lancaster & Belyea, 2006; Dolédec et al., 2007). Thus, the taxonomy resolution achieved in a study can be determinant to understand the relation of the local macroinvertebrate assemblages with hydraulics. Detailed studies with high taxonomic resolution are scarce and usually focus only on one group of macroinvertebrates (Collier, 1993; Ruse, 1995; Syrovatka et al., 2009). Usually, in most of the studies on hydraulic preferences of the macroinvertebrate community the groups of Chironomidae and Oligochatea have been only identified at family or tribe level (Mérigoux & Dolédec, 2004; Dolédec et al., 2007; Buffagni et al., 2010) due to the high taxonomy effort they require. Therefore, their specific requirements at genus or species level are unkown.

In addition, macroinvertebrates have been considered as indicators for the assessment of anthropogenic hydrological alterations (Gore, 2001; Suren & Jowett, 2006; James & Suren, 2009; Dunbar et al., 2010). Hence, the quantification of their distribution patterns according to the hydraulic conditions is essential. Although several studies defining the hydraulic habitat preferences have been done (Mérigoux & Dolédec, 2004; Dolédec et al., 2007; Buffagni et al., 2010), the knowledge of hydraulic preferences of invertebrates in large rivers is still limited (Mérigoux et al., 2009; Blettler et al, 2008) as it is their biological trait response (Statzner & Bêche, 2010). In regulated rivers, the hydrodynamic conditions are determined by the flow released from dams which can lead to changes in habitats and communities downstream and therefore affecting macroinvertebrate composition and diversity (Poff et al., 2010). The lower Ebro River, as many of the European large rivers, is highly regulated with altered natural flow regimes that affect downstream physical dynamics (Batalla et al., 2004; Vericat & Batalla, 2006) and ecological processes (Ibáñez & Prat, 2003; Ibáñez et al., 2008). Within this context, since no data relating macroinvertebrate fauna and hydraulic

preferences has been previously obtained, the knowledge of the distribution patterns of the macroinvertebrate community is essential for future biological assessments in the lower Ebro River.

In the present study we show the results of macroinvertebrate distributional patterns and biological trait composition along different hydraulic conditions in the lower Ebro River, analyzed under the concept of niche separation (Dolédec et al., 2000) and using the fourth corner method (Legendre et al., 1997; Dray & Legendre, 2008). Moreover, we contribute to the general knowledge of invertebrate hydraulic preferences in large rivers, including Chironomidae and Oligochaeta at high taxonomic resolution, and to their biological trait response, assessing the taxonomic and functional diversity. Thus, the main objectives were: (1) to determine the spatial variation of the macroinvertebrate community and their biological trait response along a hydraulic gradient and (2) to relate structural and functional community metrics to hydraulic characteristics.

Methods

Study area and sampling

The Ebro River is located in the NE Iberian Peninsula and has a drainage basin of 85,550 km^2 with a length of 928 km. The lower part of the river (100 km from the river mouth) has a mean annual flow of 426 $m^3 \cdot s^{-1}$ and is regulated by two main hydropower dams constructed in the late 1960s: Mequinença with a capacity of 1534 hm^3, and Riba-Roja, with a capacity of 207 hm^3. The latter dam regulates the water flow of the Cinca and Segre Rivers, the largest tributaries of the Ebro. Downstream of Riba-Roja the Flix Dam is the smaller one, with a capacity of 11 hm^3.

Samples were taken in the end of June 2007 at two sites in the lower Ebro River close to each other. Site 1 and site 2 were located upstream and downstream of the town of Móra d'Ebre, respectively (Fig 1), separated only 10 Km to minimize intersite differences. The end of spring was considered to be the best sampling period because the episode of high flow in April was followed by a relatively constant flow during May and June (Fig 2), and thereby the macroinvertebrate community was sampled under quite stable flow conditions. Moreover, this sampling period would include several key univoltine

species as *Ephoron virgo* (Olivier), only present in the river in spring and summer (Cid et al., 2008).

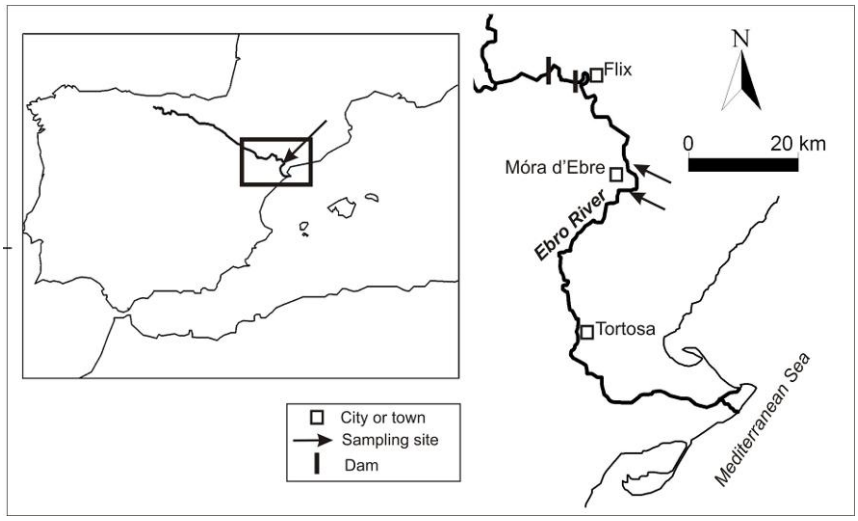


Fig.1. Sampling location in the lower Ebro River.

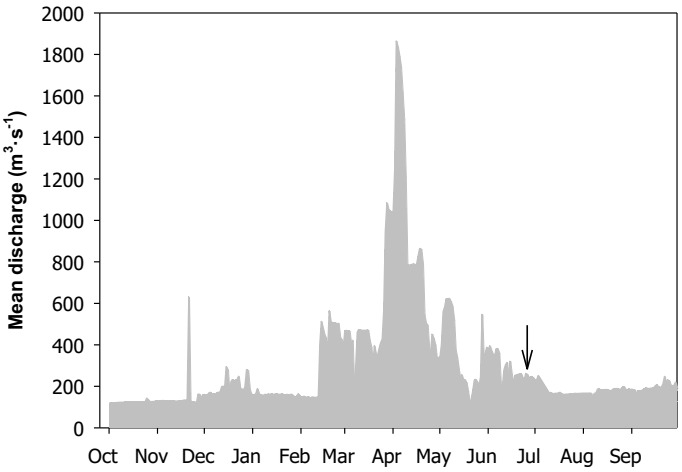


Fig.2. Mean daily discharge ($m^3 \cdot s^{-1}$) in the lower Ebro River below the dams for the hydrological year 2006-2007. Note: sampling period was in the end of June 2007 (arrow).

Since the main channel was more than 2 m deep in both sampling sites, the sampled mesohabitats were limited to the wadeable area (0-1 m) along two fluvial islands present at each site in order to collect a high variablity of the hydrodynamic conditions. Within this area, three mesohabitats (pool, run and riffle) were delimited by the mean

velocity and depth and the relative macroinvertebrate samples to be taken were assessed by the proportions occupied by each mesohabitat at each site (Table 1).

The benthic macroinvertebrates were collected using a Surber net of 50x50 cm with a mesh size of 500 μm. The samples were randomly taken and a total of 36 Surber samples were taken. The riverbed was disturbed and the subsequent sample was deposited in a tray in order to pick up attached animals from large cobbles (when present). When water velocity was zero, the sampled area corresponding to the Surber was also collected with a kick net by actively filtering the removed substrate into the net in order to not lose any organism. Afterwards invertebrate samples were preserved in 4% formaldehyde and taken to the laboratory to be identified.

Basic hydraulic measurements as depth and current velocity at 0.6 of total depth below water surfacewere were taken where the Surber net had to be placed, using a Braystoke BFM 001 current meter. Since the substrate in the sampling area is relatively homogeneous (composed mainly by pebbles, gravels and sand) we did not sample the substrate for grain size characterization at each Surber and only a sample per range of velocities was taken (see Table 1). Also the periphyton biomass (organic matter and chlorophyll a) present in the benthos were measured with 3 replicates at each range of velocities.

The dissolved oxygen (DO) in interstitial water and the % of macrophytes (%Mpht) covering the sampled area were also taken as descriptors of habitat at each Surber sample. The dissolved oxygen was measured *in situ* with a YSI 556 multiprobe by pumping the interstitial water. Since macrophytes are spread out across all hydraulic habitats in the sampling area (mainly *Potamogeton pectinatus* Linnaeus, see Ibañez et al., 2008 and Batalla & Vericat, 2009) and the Surbers were taken randomly, we obtained benthic samples with variable cover of macrophytes in order to determine if their presence could have any effect on the benthic invertebrate distribution. The % of macrophytes in the surber was calculated visually by estimating the area they occupied into the Surber area. Because we wanted to assess the effect of *Potamogeton* only in the sediment inhabiting organisms, when macrophytes were present, only sediment dwelling macroinvertebrate were taken, avoiding the analysis of macroinvertebrates living in floating plant beds. In order to do this, the floating part of the macrophyte was cut previously to the sampling and only animals present in the root part were taken.

For periphyton (chlorophyll *a* and organic matter), the methods of Steinman et al. (1996) for sampling and extraction were followed. For the sediment sampling a core sampler of 20 cm diameter similar to a cylindrical shovel was used. In the laboratory, the samples were dried and sieved with an electromagnetic sieve shaker (CISA Barcelona, model RP20), separating each particle size into the following fractions (Malavoi & Souchon, 2002): >64 mm (cobble and coarser material), 32-64 mm (coarse pebble), 16-32 mm (fine pebble), 2-8 mm (fine gravel), 0.5-2 mm (coarse sand) and 0.0625-0.5 mm (fine sand). Each particle class was weighed and from the weight of each core sample, the percentage of each size class was calculated.

Hydraulic parameters that were calculated from direct measurements included: Froude number (Fr) = $U/(g\ d)^{1/2}$, Reynolds number (Re) = $(U\ d)/v$, where d = water depth, g = acceleration due to gravity (9.8 $m^2\ s^{-1}$), U = current velocity at 0.6 depth below water surface and v = kinematic viscosity of water (0.01 $cm^2\ s^{-1}$).

Therefore, the macroinvertebrate composition was described by the following parameters: water depth, mean water velocity, Fr, Re, DO and % macrophytes in benthos. See Table 1 for mesohabitat general characteristics in the sampled area.

The identification of macroinvertebrates was done at the lowest possible taxonomic resolution, including larvae of Chironomidae and Oligochaeta, except Microcrustacea and Hydracarina which were kept at class or suborder level, respectively. The invertebrate taxa were identified according to Tachet et al. (2000), Vieira (2000) and Müller-Liebenau (1969). Oligochaetes were identified using keys in Brinkhurst (1971) and Tachet (2000). Chironomidae were identified using keys of Wiederholm (1983), Nocentini (1985), Ferrarese (1983), Rossaro (1982), Schmid (1993), Rieradevall & Brooks (2001), Heiri et al. (2004) and Brooks et al. (2007). For some taxa the presence of pupae made possible the identification at species level.

Table 1. Mesohabitat general characteristics in the sampled area. The upper part of the table shows the range of measurements included at each Surber sample and the lower part are measurements relative to each range of velocities.

	Pool		**Run**		**Riffle**	
	Site 1	**Site 2**	**Site 1**	**Site 2**	**Site 1**	**Site 2**
Surbers	n=11	n=6	n=9	n=6	n=1	n=3
U 0.6 (cm/s)	0 - 15.9	0 - 14.1	29.2 - 70.1	52.4 - 87	43.2	74.6 -85.3
Fr	0 - 0.07	0 - 0.08	0.10 - 0.32	0.19 - 0.35	0.26	0.36 - 0.48
DO (mg/l)	8.82 - 11.48	7.97 - 11.78	4.52 - 11.5	9.23 - 11.48	11.15	9.54 - 9.92
D (cm)	50 - 84	30 - 80	48 - 83	34 - 100	29	32 - 43
% Macrophyte	0 - 25	0 - 25	0 - 100	0 - 50	0	0 - 50
O.M (mg/cm^2)*	1.88 ± 0.62	1.64 ± 0.63	2.04 ± 1.31	1.44 ± 1.07	3.53 ± 3.26	3.9 ± 0.75
%O.M*	14.92 ± 1.46	20.56 ± 3.62	15.64 ± 1.92	19.85 ± 5.95	18.91 ± 2.93	20.14 ± 3.46
Chl a ($\mu g/cm^2$)*	8.43 ± 2.05	5.81 ± 0.94	3.20 ± 1.65	4.28 ± 1.01	9.57 ± 10.14	8.15 ± 2.48
Cobble and coarser material (>64 mm)	7.63	0.00	0.00	11.56	21.00	12.46
Coarse pebble (32-64mm)	14.33	48.88	0.00	34.01	23.91	37.08
Fine pebble (16-32mm)	26.37	39.86	40.82	36.43	26.81	40.07
Coarse gravel (8-16 mm)	24.34	8.28	44.59	15.96	13.76	7.97
Fine gravel (2-8 mm)	17.95	0.78	7.67	1.96	6.76	0.63
Coarse sand (0,5-2 mm)	3.92	0.29	0.19	0.03	1.04	0.37
Fine sand (0,0625-0,5 mm)	5.47	1.90	6.74	0.05	6.72	1.41

* 3 replicates were taken at each range of velocities.

Biological traits

Eleven biological traits and 62 categories obtained from a published database (Tachet et al., 2000) were used for the functional characterization of the macroinvertebrate community (Table 1). The traits in this database have an affinity score assigned for each taxa ranging from 0 to 5, from null affinity to high affinity, respectively (Chevenet et al., 1994).

When the taxonomic level of identification was higher than the level available for trait information in the database, the level available for the traits was assigned for that taxa (e.g. *Thienemaniella* sp. for Orthocladiinae, *Hemerodromia* sp. for Hemerodromiinae). When taxa identified were not present in the trait database they were excluded from the analysis. This was the case, for instance, of microcrustaceans (Copepoda, Ostracoda, Cladocera) and early larval stages of unidentified Hydroptilidae. Taxa recorded only in one sample and with abundance lower than 0.1 % of total abundance were omitted from the analysis.

Table 1. Biological traits and categories for macroinvertebrates present in this study (see Tachet et al., 2000).

Biological traits	**Category**
Maximal size	<0.25 cm
	>0.25–0.5 cm
	>0.5–1 cm
	>1–2 cm
	>2–4 cm
	>4–8 cm
	>8 cm
Life cycle duration	<1 year
	>1 year
Potential no. reproductive cycles per year	<1
	1
	>1
Aquatic stages	Egg
	Larva
	Nymph
	Imago
Reproduction type	Ovoviviparity
	Isolated eggs, free
	Isolated eggs, cemented
	Clutches, cemented or fixed
	Clutches, free
	Clutches, in vegetation
	Clutches, terrestrial
	Asexual reproduction
Dispersal	Aquatic passive
	Aquatic active
	Aerial passive
	Aerial active
Resistance form	Eggs, statoblasts
	Cocoons
	Cells against desiccation
	Diapause or dormancy
	None
Respiration	Tegument
	Gill
	Plastron
	Spiracle (aerial)
Locomotion and substratum relation	Flier
	Surface swimmer
	Full water swimmer
	Crawler
	Burrower (epibenthic)
	Interstitial (endobenthic)
	Temporarily attached
	Permanently attached
Food	Fine sediment and microorganisms
	Detritus < 1 mm
	Plant detritus > 1 mm
	Living microphytes
	Living macrophytes
	Dead animal > 1 mm
	Living microinvertebrates
	Living macroinvertebrates
	Vertebrates
Feeding habits	Absorber
	Deposit feeder
	Shredder
	Scraper
	Filter feeder
	Piercer (plants or animals)
	Predator (carver/engulfer/swallower)
	Parasite

Data analyses

The relation of environmental variables with the macroinvertebrate fauna was explored comparing a taxa-density dataset with a hydraulics dataset including water velocity, Re, Fr and depth corresponding to each Surber sample. Hydraulic data and macroinvertebrate abundances were log transformed. These two datasets were used to perform the Outlying Mean Index analysis (OMI) (Dolédec et al., 2000), following the methods described in Mérigoux & Dolédec (2004). This is a multivariate method which analyses "niche separation" and "niche breadth" of species in a community assemblage. The resulting OMI values represent quantification of the marginality or tolerance of each taxa to the measured environmental variables, that is, the higher is the value the higher the marginality. We considered the OMI analysis since it has been used before to describe the habitat preference of species along environmental gradients (Malard et al., 2003; Mérigoux & Dolédec, 2004; Thuiller et al., 2004) and because, in comparison to other widely used multivariate methods, OMI gives equal weighting to samples whether they are poor or rich in species, and because it does not assume an *a priori* response curves of species thus it can describe unimodal (according to a Canonical correspondence analysis, CCA) or linear (according to a Redundancy analysis, RDA) response curves to the environment. In order to test the statistical significance of the marginality for each taxon, a random permutation test (Monte Carlo test, 1000 permutations) was used. The permutation test checks if the mean position of each taxa according to the environmental gradient is different from a theoretical general mean (Dolédec et al., 2000).

The Fourth Corner method analysis (Legendre et al., 1997; Dray & Legendre, 2008) was performed to test the relationship of each macroinvertebrate trait category with the environmental variables. This method uses different permutation models depending on the hypothesis to be tested. We used the Model 5 because is strictly equivalent to RLQ method proposed by Dolédec et al. (1996) which uses three tables: R (samples x environmental variables), L (samples x taxa) and Q (taxa x trait categories). The first two tables (R and L) correspond to those previously used in the OMI analysis and the third one (Q) is the table corresponding to the affinity of each taxa for each trait category (see Biological traits section). The output of the analysis is a Pearson product-moment correlation coefficient (r) for each environmental variable used with its associated *P*-value obtained by random permutations (999 runs).

In order to determine the taxonomic and trait diversity along the hydraulic gradient, the taxon and trait richness and Shanon-Wiener, Simpson and Rao diversity indices were used. The Rao index (Champely & Chessel, 2002; Bady et al., 2005; Bonada et al., 2007) is calculated by using the dissimilarity matrix (measured, for instance, as Euclidean distance) of the dataset of relative abundance of species or traits per sample (see above explanation for this dataset). In order to obtain the dataset of relative abundance of traits per sample, the affinity of each taxon for each trait category was multiplied by the taxon density abundance (see Chevenet et al., 1994; Mérigoux & Dolédec, 2004; Bonada et al., 2007). Linear regressions were performed to fit the relationship between hydraulics and diversity.

All the statistical multivariate analysis and graphs were carried out using R free software (Ihaka & Gentleman, 1996) with ADE4 (Thioulouse et al., 1997) and Vegan (Oksanen et al., 2010) libraries.

Results

Macroinvertebrate abundance and community metrics

A sum of 275,128 individuals of 116 macroinvertebrate taxa were collected in this study. The predominant taxa corresponded to the Chironomids of the subfamily Orthocladiinae representing the 40.6 % of the total abundance (e.g. *Cricotopus vierriensis* grp. and *C. (C.) bicinctus* (Meigen), representing a 13.71% and 13.15 %, respectively). The Amphipoda *Echinogammarus longisetosus* Pinkster was the most abundant species with a 20.75 %, followed by *Baetis fuscatus* (L.) with a 17.39%. Within Oligochaeta, the species *Stylaria lacustris* (L.) was the most representative (1.17% of total macroinvertebrate abundance) and within Trichoptera *Hydropsyche exocellata* Duföur (1.56%). Of the 116 taxa obtained, 28 were taken out of the analysis due to their low abundance and sample occurrence (see SI 7 for details). These minor taxa were some Naididae (e.g. *Dero digitata* [Müller]), several Chironomidae (e.g. *Parametriocnemus stylatus* [Kieffer], *Eukiefferiella gracei* [Edwards]) and other Dipterans (e.g. *Atrichops crassipes* Meigen), Coleoptera (*Pomatinus substriatus* [P.H. Müller] and *Potamophilus* sp.), Odonata (*Gomphus* sp.) and Heteroptera (*Naucoris maculates* Fabricius).

The total density of macroinvertebrates was significantly related with the water velocity (linear regression model, p-value>0.05), with higher densities at higher water velocities (Figure 3). All the diversity measures (richness, Shannon, Simpson, Rao diversity, trait richness, trait Shannon diversity, trait Simpson diversity and trait Rao diversity) were correlated with water velocity, showing a significant inverse linear relationship (Figure 4).

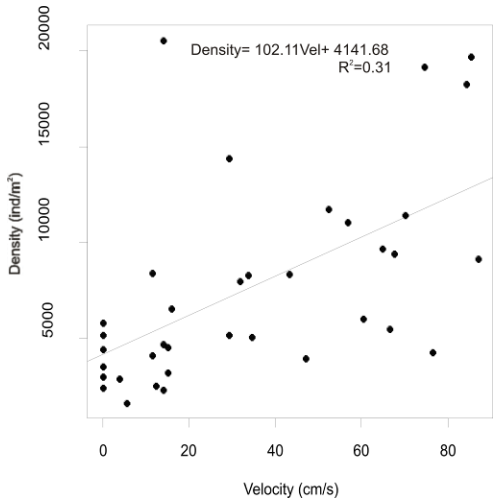


Fig 3. Relationship between density of macroinvertebrates (ind/m^2) and water velocity (cm/s).

Macroinvertebrate niche separation: OMI analysis

The results of the OMI analysis (niche separation) with the macroinvertebrate community showed the position of each taxon according to the hydraulic gradient with a measure of the marginality and tolerance. The first and second axis of the ordination analysis explained 75.77% and 11.31% of the variability, respectively, being the weight of water velocity, Re and Fr the most important parameters (see SI 1 for details of the analysis). Although DO in sediment and % of macrophytes in the riverbed were not the main factors explaining the macroinvertebrate distribution, they presented opposite scores in the second axis. The global OMI analysis was statistically significant (Monte Carlo test, P-value=0.001) and 38 taxa over 87 presented significant hydraulic marginality (Table 1). Concretely, the family of Chironomidae presented a wide range of hydraulic preferences, with differences within the same subfamily, tribe or genus. On

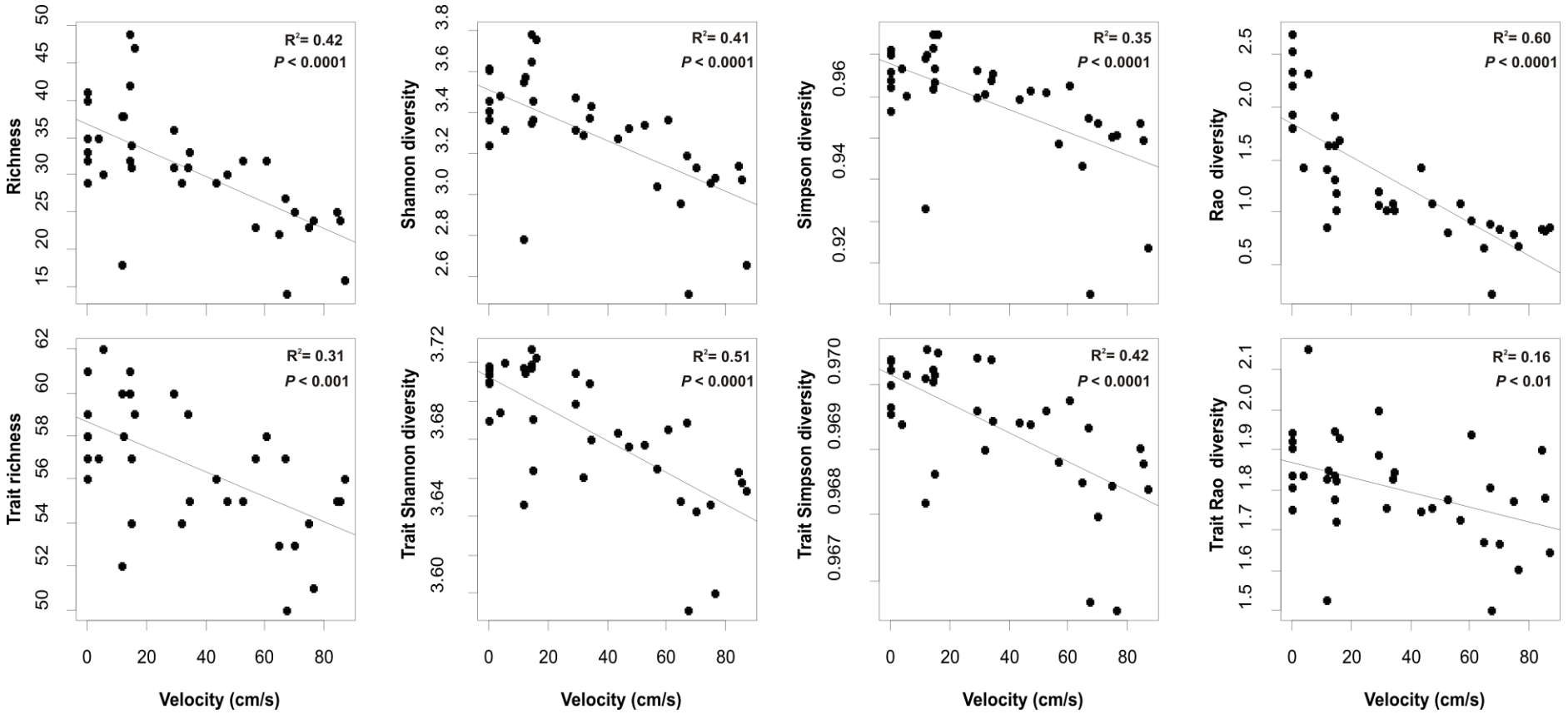


Figure 4. Relationship between different diversity measures along the velocity gradient. All linear regressions were significant.

the other hand, Oligochaeta tended to occupy areas of low water velocity, except for the Lumbricid *Eiseniella tetraedra*.

The most abundant species as *Baetis fuscatus*, *B. pavidus*, *Hydropsyche exocellata*, *Echinogammarus longisetosus*, *Theodoxus fluviatilis*, *Dugesia sicula* and the Chironomids *Synorthocladius semivirens* and *Cricotopus (Cricotopus) vierriensis* grp. presented a significant marginality, showing habitat preferences for high water velocities (Figure 6). Species with lower abundances but also with significant preferences for high water velocities were the Chironomids *Rheocricotopus (Psilocricotopus) fuscipes*, *Eukiefferiella minor-fittkaui*, *Rheocricotopus (Ps.) chalybeatus*, *Cricotopus (C.) trifascia*. Both *Rheocricotopus (Ps.) chalybeatus* and *Cricotopus (C.) trifascia* had the lowest tolerance, which implies that they appeared to be the most specialist species for high water velocity in our study.

Abundant species presenting significant marginality but having low tolerance for high water velocity were the group of *Cricotopus (Isocladius) sylvestris*, the ephemeropterans *Caenis luctuosa* and *Choroterpes picteti*, and the Ostracoda group. In general, though not being present at very high abundances, the highest OMI values were obtained for species with preferences for low water velocity, showing very high habitat marginality and very low tolerance values. For instance, this was the case of the Naidid *Nais* cf. *barbata*, the Chironomids *Polypedilum (Tripodura) scalaenum* grp. and *Tanytarsus pallidicornis* type (sensu Heiri et al., 2001), the Ephemeroptera *Cloeon dipterum* and the group of Cladocera.

Taxa showing no significant marginality, as the Tubificid *Potamothrix bavaricus*, the Chironomid *Cricotopus (C.) bicinctus* or the Asian clam *Corbicula fluminea*, showed a preference for intermediate water velocities; however, they were distributed along all the hydraulic conditions (see Table 2).

Table 2. Niche parameters of the macroinvertebrate community of the lower Ebro River related to hydraulics: the inertia, the outlying mean index (OMI), the tolerance index (Tol), and the residual tolerance index (RTol). Values in italics represent the corresponding percentages of variability. Num is the number of random permutations out of 1000 needed to obtain a higher value than the observed OMI. Taxa showing significant marginality are in bold.

Taxa	**inertia**	**OMI**	**Tol**	**Rtol**	***omi***	***tol***	***rtol***	**Num**	**Pvalue**
Hydra sp.	7.56	1.32	3.72	2.51	17.50	49.2	33.3	276	0.271
***Dugesia sicula* Lepori**	**6.38**	**0.03**	**2.13**	**4.22**	**0.40**	**33.3**	**66.2**	**4**	**0.004**
Prostoma graecense Böhmig	5.63	0.13	0.76	4.74	2.20	13.6	84.2	518	0.526
Mermithidae	**8.34**	**1.96**	**2.43**	**3.96**	**23.50**	**29.1**	**47.4**	**16**	**0.021**
Potamothrix bavaricus (Oschmann)	7.53	0.30	0.95	6.28	4.00	12.6	83.4	978	0.976
Potamothrix hammoniensis (Michaelsen)	2.60	1.40	0.00	1.19	54.00	0	46	761	0.739
Tubificidae SSC	**8.30**	**1.79**	**3.38**	**3.12**	**21.60**	**40.8**	**37.6**	**41**	**0.033**
***Branchiura sowerbyi* Beddard**	**7.68**	**4.43**	**1.87**	**1.37**	**57.70**	**24.4**	**17.9**	**3**	**0.008**
Lumbriculidae	8.21	0.78	1.83	5.59	9.60	22.3	68.1	462	0.467
Nais cf. barbata	**13.56**	**12.28**	**0.01**	**1.27**	**90.50**	**0.1**	**9.4**	**14**	**0.025**
Nais bretscheri Michaelsen	4.99	0.19	0.60	4.20	3.90	12.1	84.1	944	0.950
Nais cf. bretscheri	**7.08**	**1.35**	**2.69**	**3.05**	**19.00**	**37.9**	**43**	**18**	**0.018**
Ophidonais serpentina (Müller)	5.18	1.68	1.46	2.04	32.50	28.1	39.4	346	0.376
***Stylaria lacustris* (Linnnaeus)**	**6.68**	**0.99**	**2.15**	**3.54**	**14.80**	**32.2**	**53**	**0**	**0.001**
Slavina appendiculata (Udeken)	7.76	1.22	2.99	3.55	15.80	38.6	45.7	485	0.484
Vejdovskyella intermedia (Bretscher)	8.33	2.85	2.45	3.03	34.20	29.5	36.4	253	0.244
***Eiseniella tetraedra* (Savigny)**	**11.04**	**2.25**	**2.76**	**6.03**	**20.40**	**25**	**54.6**	**41**	**0.040**
Erpobdella sp.	3.77	2.63	0.22	0.92	69.80	5.9	24.3	488	0.468
Piscicola geometra (L.)	5.78	0.40	0.92	4.45	6.90	16	77.1	451	0.407
Corbicula fluminea (O.F. Müller)	5.86	0.03	0.85	4.98	0.60	14.4	85	453	0.472
Dreissema polymporpha (Pallas)	4.99	0.35	1.79	2.85	7.10	35.8	57.1	194	0.230
Ferrissia (Pettancylus) clessiniana (Jickeli)	2.14	1.41	0.06	0.67	65.60	3	31.4	775	0.779
Physella (Costatella) acuta (Draparnaud)	5.91	0.08	0.95	4.88	1.40	16.1	82.5	739	0.748
Radix sp.	4.45	0.52	0.53	3.40	11.60	12	76.4	627	0.663
***Theodoxus fluviatilis* (L.)**	**5.41**	**0.14**	**2.00**	**3.27**	**2.50**	**37**	**60.5**	**5**	**0.009**
Hydracarina	5.84	0.12	0.69	5.03	2.10	11.8	86.1	140	0.137
Cladocera	**12.79**	**12.66**	**0.00**	**0.13**	**99.00**	**0**	**1**	**0**	**0.001**
Copepoda	**7.85**	**1.90**	**2.81**	**3.14**	**24.20**	**35.7**	**40**	**7**	**0.012**
Ostracoda	**7.13**	**0.98**	**2.45**	**3.70**	**13.70**	**34.4**	**51.9**	**0**	**0.001**
Caridean larvae	**5.75**	**1.83**	**1.42**	**2.50**	**31.80**	**24.8**	**43.5**	**14**	**0.007**
***Echinogammarus longisetosus* Pinkster**	**5.98**	**0.11**	**2.40**	**3.47**	**1.80**	**40.1**	**58**	**0**	**0.001**
Proasellus meridianus (Racovitza)	6.03	0.07	1.09	4.87	1.10	18.1	80.8	979	0.966
***Baetis fuscatus* (L.)**	**5.50**	**0.32**	**1.75**	**3.43**	**5.70**	**31.9**	**62.4**	**0**	**0.001**
***Baetis pavidus* Grandi**	**5.60**	**0.82**	**1.06**	**3.72**	**14.60**	**19**	**66.4**	**0**	**0.001**
***Caenis luctuosa* (Burmeister)**	**6.38**	**0.29**	**2.84**	**3.25**	**4.50**	**44.6**	**50.9**	**2**	**0.006**
Choroterpes picteti (Eaton)	6.63	0.14	3.46	3.03	2.10	52.2	45.7	65	0.066
***Cloeon dipterum* (L.)**	**13.56**	**12.59**	**0.03**	**0.94**	**92.80**	**0.3**	**6.9**	**15**	**0.010**
***Cloeon simile* Eaton**	**9.78**	**4.47**	**3.96**	**1.35**	**45.70**	**40.5**	**13.8**	**68**	**0.072**
Ephoron virgo (Olivier)	5.46	0.17	2.14	3.16	3.10	39.1	57.8	389	0.414
Pseudocloeon atrebatinus (Eaton)	6.13	0.75	0.83	4.55	12.30	13.5	74.2	183	0.180
Platycnemis sp.	6.63	1.28	1.01	4.34	19.30	15.2	65.5	105	0.096
Zigoptera (immature)	6.47	2.86	0.14	3.48	44.10	2.1	53.8	437	0.450
Hydrometra sp.	4.51	1.09	0.52	2.90	24.20	11.5	64.3	873	0.892
***Micronecta* sp.**	**7.94**	**2.12**	**3.74**	**2.09**	**26.70**	**47.1**	**26.3**	**1**	**0.001**
Collembola	7.20	2.55	2.04	2.62	35.40	28.3	36.3	238	0.238

Continued from Table 2

Taxon	**inertia**	**OMI**	**Tol**	**Rtol**	***omi***	***tol***	***rtol***	**Num**	**Pvalue**
Aulonogyrus sp.	5.24	2.00	2.58	0.66	38.20	49.3	12.5	587	0.600
Dryops sp.	5.41	1.28	0.41	3.72	23.70	7.5	68.8	482	0.473
Hydaticus sp.	10.01	4.39	0.12	5.50	43.80	1.2	55	171	0.189
Laccophilus sp.	7.01	0.74	1.86	4.41	10.60	26.5	62.9	242	0.247
Ceraclea dissimilis (Stephens)	12.17	3.24	3.87	5.06	26.60	31.8	41.6	78	0.064
Ceraclea sobradieli (Navás)	6.96	0.34	1.08	5.54	4.80	15.5	79.6	615	0.651
***Ecnomus tenellus* (Rambur)**	**4.83**	**1.69**	**0.94**	**2.19**	**35.10**	**19.5**	**45.4**	**33**	**0.026**
***Hydropsyche exocellata* Duföur**	**5.96**	**0.40**	**1.87**	**3.69**	**6.60**	**31.4**	**62**	**0**	**0.001**
Hydroptila sp.	5.64	0.02	1.13	4.48	0.40	20.1	79.5	239	0.247
Hydroptilidae stage 1-4	6.01	0.06	0.94	5.02	0.90	15.6	83.5	617	0.554
Mystacides azurea (L.)	6.02	2.17	1.84	2.01	36.00	30.5	33.4	172	0.194
***Orthotrichia angustella* (McLachlan)**	**7.11**	**2.65**	**2.64**	**1.82**	**37.30**	**37.1**	**25.6**	**0**	**0.001**
Psychomia pusilla (Fabricius)	5.16	0.08	0.87	4.22	1.50	16.8	81.7	735	0.697
***Ablabesmyia longistyla* Fittkau**	**5.63**	**0.91**	**2.11**	**2.61**	**16.10**	**37.5**	**46.4**	**6**	**0.003**
***Procladius* sp.**	**10.36**	**7.55**	**2.11**	**0.70**	**72.90**	**20.4**	**6.7**	**4**	**0.011**
Thienemannimyia sp.	5.62	0.36	1.23	4.03	6.40	21.9	71.7	434	0.432
Potthastia gaedii (Meigen)	5.01	1.66	0.80	2.56	33.10	15.9	51	67	0.057
***Cricotopus (I.) sylvestris* grp.**	**8.07**	**1.34**	**4.02**	**2.71**	**16.60**	**49.8**	**33.6**	**0**	**0.004**
Cricotopus (C.) albiforceps (Kieffer)	2.64	1.21	0.37	1.06	45.70	14.2	40.1	174	0.152
Cricotopus (C.) bicinctus (Meigen)	6.07	0.01	1.80	4.27	0.20	29.6	70.2	166	0.187
***Cricotopus (C.) trifascia* Edwards**	**4.48**	**2.21**	**0.38**	**1.89**	**49.30**	**8.5**	**42.1**	**2**	**0.004**
***Cricotopus (C.) vierriensis* grp.**	**5.84**	**0.02**	**2.21**	**3.61**	**0.40**	**37.8**	**61.8**	**15**	**0.018**
Eukiefferiella minor-fittkaui	**5.29**	**0.53**	**1.90**	**2.86**	**10.10**	**35.9**	**54**	**29**	**0.013**
Orthocladius (O.) obumbratus Johansen	6.51	1.11	2.90	2.50	17.00	44.5	38.5	104	0.088
Paratrichocladius rufiventris (Meigen)	6.05	0.72	0.84	4.49	12.00	13.9	74.2	490	0.490
***Rheocricotopus (Ps.) chalybeatus* (Edwards)**	**6.12**	**1.27**	**0.59**	**4.26**	**20.70**	**9.6**	**69.6**	**0**	**0.001**
***Rheocricotopus (Ps.) fuscipes* (Kieffer)**	**5.91**	**0.35**	**1.60**	**3.96**	**5.90**	**27.1**	**67**	**19**	**0.032**
***Synorthocladius semivirens* (Kieffer)**	**5.08**	**0.36**	**1.09**	**3.63**	**7.00**	**21.4**	**71.6**	**0**	**0.001**
***Thienemanniella flaviforceps* (Kieffer)**	**6.24**	**0.63**	**1.77**	**3.84**	**10.10**	**28.4**	**61.6**	**9**	**0.015**
***Cryptochironomus* sp.**	**7.48**	**1.98**	**3.63**	**1.87**	**26.50**	**48.5**	**25**	**23**	**0.014**
***Dicrotendipes nervosus* grp.**	**9.63**	**6.50**	**2.35**	**0.78**	**67.50**	**24.4**	**8.1**	**15**	**0.023**
Polypedilum (Polypedilum) nubifer (Skuse)	7.40	4.01	1.65	1.73	54.20	22.4	23.4	190	0.195
***Polypedilum (Tripodura) scalaenum* grp.**	**10.86**	**8.41**	**1.86**	**0.58**	**77.50**	**17.2**	**5.4**	**4**	**0.010**
Harnischia sp.	4.83	0.92	0.17	3.73	19.10	3.6	77.3	212	0.236
***Polypedilum* type A (sensu Brooks et al., 2007)**	**8.37**	**2.66**	**4.31**	**1.39**	**31.80**	**51.6**	**16.6**	**6**	**0.009**
Cladotanytarsus sp.	6.04	1.18	1.05	3.81	19.60	17.4	63.1	488	0.492
Rheotanytarsus sp.	6.39	0.84	1.43	4.13	13.10	22.3	64.6	104	0.097
Tanytarsus chinyensis grp.	4.29	1.83	0.41	2.05	42.80	9.5	47.7	585	0.596
***Tanytarsus pallidicornis* type (sensu Heiri et al., 2001)**	**12.44**	**12.24**	**0.00**	**0.20**	**98.40**	**0**	**1.6**	**29**	**0.017**
Virgatanytarsus sp.	6.37	0.83	2.75	2.79	13.00	43.2	43.8	164	0.135
Hemerodromia sp.	7.01	1.12	0.77	5.13	15.90	11	73.1	714	0.756
***Simulium erytrocephalum* (De Geer)**	**7.13**	**0.82**	**2.14**	**4.16**	**11.60**	**30.1**	**58.4**	**14**	**0.015**

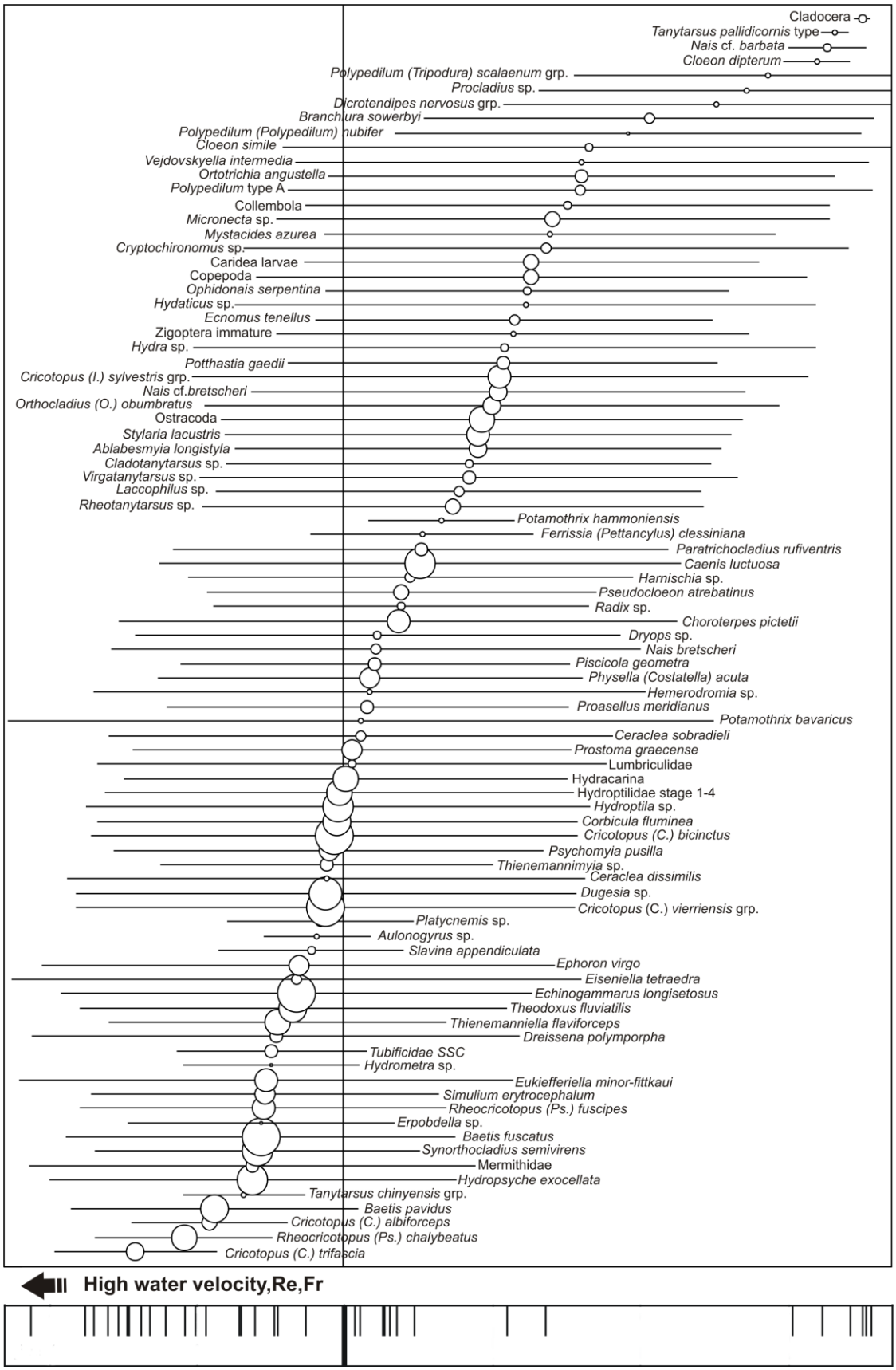


Figure 6. Position of niche taxa on the first OMI sample scores for macroinvertebrate assemblages in the lower Ebro River along the hydraulic gradient. The position of each taxa (dots) corresponds to the weighted mean of their distribution in sites and the horizontal lines are the standard deviation representing the niche amplitude. The size of each dot is relative to the abundance. The bottom vertical bars correspond to the Surber sample scores along the gradient, with increasing water velocity, Re and Fr from right to left. The vertical line across the panel is the theoretical position of a hypothetical species present at all the habitat gradient.

Biological traits and habitat constraints: Fourth corner method

Of the total 62 trait categories analyzed, 32 presented significant r-values for mean velocity and Froude number and 29 for Reynolds number. Fewer relationships were obtained for depth, DO and % macrophyte in sediment, with 16, 13 and 14 significant trait categories, respectively (Table 3). Thus, the functional characteristics of taxa were mainly influenced by the variables regarding hydraulics. Out of the 11 analyzed traits only the life cycle duration was not influenced by the hydraulic conditions but appeared to be influenced by the DO in sediment. The maximal size, potential number of reproductive cycles per year, the aquatic stages, the reproduction type, dispersal, resistance form, respiration, locomotion and substratum relation, food and feeding habits showed a significant relationship with the hydraulics at least in one of their categories.

Among those traits presenting significant r-values, several trait categories were correlated with high values of current velocity, Re and Fr. These traits were a body size between 2 and 4 cm, one potential reproductive life cycle per year, aquatic stages as egg, a reproduction by ovoviviparity or clutches cemented or fixed, with no resistance forms or if present as eggs or statoblasts, and respiration by gills. Locomotion is characterized by crawlers and organisms temporarily attached, and food and feeding habits were correlated with filter-feeders and scrapers and feeding on coarse plant detritus and dead animals. In contrast, the smallest organisms (< 0.5 cm) and those having a higher potential body size (>8 cm) were negatively correlated with current velocity, Re and Fr. The lower the values of this variables, the higher the proportion of organisms with more than one reproductive cycle per year, aquatic stages as larva, a reproduction by free isolated eggs, free clutches or asexual, an aerial passive dispersal, cocoons as resistance form and respiration by the tegument, plastron or spiracle. Full water swimmers, burrowers and interstitial organisms followed the same pattern, as well as deposit feeders and piercers.

Although depth, DO and % macrophytes were not correlated with many trait categories some were significant. DO in sediment was negatively correlated with those organisms with a body size between 4-8 cm, those with a lifespan more than one year, clutches in vegetation or asexual reproduction, aquatic passive dispersal, cocoons as resistant form, living in the interstices and feeding as absorbers, deposit feeders and parasites. Those

traits positively correlated with the % of macrophyte in sediment were medium-large sizes, one reproductive life cycle per year, reproduction by clutches cemented or fixed or clutches in vegetation, breathing by spiracle, moving as a flier or being temporarily atached and feeding on living macroinvertebrates.

Table 3. Results from the fourth-corner method (Model 5) using macroinvertebrate density, biological traits and habitat variables. The numbers presented are the r-values corresponding to the correlation of each trait category with the habitat. The level of significance is indicated with * (P<0.05), ** (P<0.01) or ***(P<0.001). Those P-values nearly significant are indicated with '(P<0.1).

Trait	Category	U (cm/s)	Fr	Re	D (m)	DO (mg/l)	%Mpht
Maximal size	<0.25 cm	**-0.115*** **	**-0.114*** **	**-0.108** **	-0.008	-0.003	**-0.046***
	>0.25–0.5 cm	**-0.095** **	**-0.100** **	**-0.078** **	0.023	0.029'	-0.019
	>0.5–1 cm	0.015	0.013	0.009	-0.032'	0.025	-0.027
	>1–2 cm	0.009	0.008	0.023	**0.053** **	-0.019	**0.044***
	>2–4 cm	**0.086*** **	**0.096*** **	**0.052***	**-0.057** **	0.017	-0.009
	>4–8 cm	0.010	0.004	0.021	0.015	**-0.064** **	**0.036***
	>8 cm	**-0.069** **	**-0.063***	**-0.063***	**0.065** **	-0.004	**-0.042***
Life cycle duration	<1 year	-0.006	-0.005	-0.011	-0.017	**0.051***	-0.027
	>1 year	0.006	0.005	0.011	0.017	**-0.051***	0.027
Potential no.	<1	0.004	0.002	0.034'	**0.063** **	-0.021	0.032'
reproductive cycles	1	**0.060** **	**0.058** **	**0.059** **	0.002	0.015	**0.046***
per year	>1	**-0.060** **	**-0.058** **	**-0.062** **	-0.009	-0.012	**-0.048***
Aquatic stages	Egg	**0.094*** **	**0.097*** **	**0.091*** **	-0.003	-0.016	0.022
	Larva	**-0.048***	**-0.047***	**-0.062** **	-0.023	0.000	-0.014
	Pupa	-0.033'	-0.034'	-0.036'	-0.006	0.033'	-0.012
	Adult	0.002	0.000	0.017	0.026	-0.025	0.007
Reproduction type	Ovoviviparity	**0.059***	**0.064** **	0.034'	**-0.048***	0.018	-0.017
	Isolated eggs, free	**-0.041***	-0.038'	-0.038'	0.035'	0.006	-0.001
	Isolated eggs, cemented	-0.036'	-0.033'	-0.026	0.032'	0.013	-0.038'
	Clutches, cemented or fixed	**0.075** **	**0.070** **	**0.069** **	-0.033'	-0.014	**0.040***
	Clutches, free	**-0.108*** **	**-0.112*** **	**-0.093*** **	0.030'	0.028	-0.009
	Clutches, in vegetation	-0.005	-0.012	0.020	0.032'	**-0.043***	**0.050***
	Clutches, terrestrial	0.018	0.018	0.010	-0.019	0.018	0.008
	Asexual reproduction	**-0.079** **	**-0.079** **	**-0.063***	**0.043***	**-0.050***	-0.014
Dispersal	Aquatic passive	-0.009	-0.008	-0.001	0.014	**-0.046***	-0.023
	Aquatic active	0.026	0.026	0.023	-0.001	0.018	0.011
	Aerial passive	**-0.063** **	**-0.064** **	**-0.064** **	0.003	0.034'	-0.004
	Aerial active	0.035	0.034'	0.025	-0.025	0.021	0.026
Resistance form	Eggs, statoblasts	**0.062** **	**0.065** **	**0.042***	-0.032'	0.007	0.023
	Cocoons	**-0.101*** **	**-0.104*** **	**-0.069** **	**0.069** **	**-0.042***	-0.022
	Cells against desiccation	0.018	0.019	0.020	0.021	0.003	0.004
	Diapause or dormancy	-0.019	-0.022	-0.003	0.007	0.000	0.014
	None	**0.053** **	**0.056** **	0.029'	**-0.043***	0.029'	-0.007
Respiration	Tegument	**-0.110*** **	**-0.116*** **	**-0.069** **	**0.082*** **	-0.027	-0.001
	Gill	**0.129*** **	**0.140*** **	**0.085*** **	**-0.065** **	0.035'	-0.018
	Plastron	**-0.090** **	**-0.086** **	**-0.084** **	0.015	0.009	-0.019
	Spiracle (aerial)	-0.031'	**-0.047***	-0.020	**-0.053***	-0.030'	**0.065** **

Continued from Table 3

Trait	Category	U (cm/s)	Fr	Re	D (m)	DO (mg/l)	%Mpht
Locomotion and substratum relation	Flier	-0.031'	**-0.038***	-0.017	0.011	0.007	**0.046***
	Surface swimmer	0.009	0.006	0.004	-0.028	0.011	0.015
	Full water swimmer	**-0.056****	**-0.059****	**-0.046***	0.015	0.013	-0.012
	Crawler	**0.067****	**0.069****	**0.054***	**-0.040***	0.021	-0.012
	Burrower (epibenthic)	**-0.040***	-0.037'	-0.035'	**0.044***	0.010	-0.009
	Interstitial (endobenthic)	**-0.053****	**-0.055****	**-0.036***	**0.039***	**-0.070****	-0.015
	Temporarily attached	**0.051***	**0.049***	**0.040***	-0.026	-0.020	**0.051****
	Permanently attached	0.015	0.016	0.007	-0.018	0.030'	-0.023
Food	Fine sediment + microorganisms	**-0.111*****	**-0.119*****	**-0.089*****	0.006	**-0.068****	-0.005
	Detritus < 1 mm	**-0.047***	**-0.044***	**-0.055***	0.002	-0.011	0.010
	Plant detritus > 1 mm	**0.082*****	**0.085*****	**0.066****	**-0.045***	0.016	-0.010
	Living microphytes	0.010	0.014	0.012	0.023	-0.006	-0.043
	Living macrophytes	-0.030'	-0.025	-0.034'	0.011	**0.054****	-0.055
	Dead animal > 1 mm	**0.071****	**0.069****	**0.074****	-0.011	0.036'	-0.006
	Living microinvertebrates	0.006	0.007	0.003	0.006	0.012	0.004
	Living macroinvertebrates	0.015	0.010	0.017	-0.011	-0.016	**0.046***
	Vertebrates	-0.016	-0.023	0.008	0.017	-0.024	0.025
Feeding habits	Absorber	-0.023	-0.027	-0.016	0.016	**-0.095*****	0.025
	Deposit feeder	**-0.128*****	**-0.129*****	**-0.110*****	**0.045***	**-0.039***	**-0.040***
	Shredder	0.013	0.017	0.000	-0.034'	0.017	-0.022
	Scraper	0.033'	0.033'	**0.042***	0.008	0.016	-0.018
	Filter feeder	**0.091*****	**0.094*****	**0.066****	-0.033'	0.010	0.036'
	Piercer (plants or animals)	**-0.085*****	**-0.089*****	**-0.072*****	0.008	0.029'	-0.021
	Predator (carver/engulfer/swallower)	0.005	0.004	0.012	0.020	0.002	0.010
	Parasite	0.013	0.011	0.014	-0.011	**-0.042***	**0.083****

Discussion

Hydraulic conditions as determinant of the macroinvertebrate community composition

Within the habitat parameters analyzed, the mean water velocity was the main explanatory variable in this study, followed by Fr and Re. Thereby the hydraulic conditions determined the macroinvertebrate distribution, which was in accordance to previous studies defining the invertebrate preferences from lentic to lotic habitats (Quinn & Hickey, 1994; Rempel et al., 2000; Mérigoux & Dolédec, 2004; Mérigoux et al., 2009; Sagnes et al., 2008; Buffagni et al., 2010). Because there is a relationship between the water column velocity and shear stress, in areas where substrate has similar characteristics the hydraulic explanatory variables can be simplified by using current velocity (Quinn & Hickey, 1994; see Syrovátka et al., 2009 and Dolédec et al., 2007).

Thus, accordingly to the relatively homogeneous substrate composition within the sampled area of the Ebro, the current velocity summarized all the other measured hydraulic variables.

Differences in diversity metrics and abundance were observed along the hydraulic gradient. The high invertebrate density at high velocity areas can be influenced by the high abundance of plocon (*Cladophora* sp.) that could provide food for scraper taxa such as *Cricotopus* spp. and increase the substratum to be colonized by filter feeder species like *Hydropsyche*, as observed by Muñoz & Prat (1994) in high current areas of the lower Ebro. This can be related with the high nutrient load received by the Ebro River (see Ibañez et al., 2008; Sabater et al., 2008) which might have propitiated a situation where a few dominant species occupy areas of higher water velocities due to high food availability. Doisy et al. (2001) observed that the relation of macroinvertebrate density and hydraulics changed depending on the season according to the population dynamics of dominant species along the year. Accordingly, as described in other European rivers (Mérigoux & Dolédec, 2004), species as *H. exocellata* are very abundant in spring, which can directly influence the invertebrate abundance in fast flowing areas. On the contrary, a negative relationship between macroinvertebrate densities and flow were observed by Rempel et al. (1999) in the Frasier River, a large gravel bed river as the Ebro but which has natural hydrological dynamics since it is a non regulated river and the content of nutrients is lower.

The significant negative relationship between all the community metrics (either considering taxonomy or traits) and water velocity is likely due to the presence of a few dominant species with similar traits in fast flowing areas. This is consistent with the fact that the diversity indices take into account the proportion of each species or trait per sample (Shannon, Simpson and Rao diversity). When measuring richness along a hydraulic gradient, similar results were previously observed by Rempel et al. (1999) from April to September in the Frasier River and by Mérigoux & Dolédec (2004) in spring in the Ardèche River. In contrast, in a study performed in summer in two gravel-bed rivers in New Zealand (Quinn & Hickey, 1994) a positive relationship between richness and hydraulics was obtained, while others did not found any significant relationship (Gore, 1978; Doisy et al., 2001). Quinn & Hickey (1994) obtained a decline in Shannon diversity with increasing velocity in one of the studied rivers, as in the Ebro, and an increase in the other. On the other hand, Doisy et al. (2001) observed that

Simpson diversity was positively correlated with mean current velocity, Fr and Re. In many of these studies Chironomidae and Oligochaeta are not included at genus or species level and usually are the dominant taxa. In our case, even taking into account all the community at a similar taxonomic level, few species dominated in areas with high current velocity which were responsible of the lower values of diversity indexes. As mentioned by Quinn & Hickey (1994), the decline in diversity with increasing velocity in one of the studied rivers was related to the increasing dominance of Hydropsychids, same as in the Ebro for *H. exocellata*, *Baetis* sp. or *Cricotopus spp.*, and this could be one of the reasons of the discrepancies across studies relating diversity indices with hydraulics. Again, the relatively high eutrophy of the Ebro, with the subsequent availability of food, might be responsible of the decrease of diversity indexes with increasing water velocity. Regarding functional metrics, the negative relationship of all the trait diversity indexes with increasing water velocity is in disagreement with the results of Mérigoux & Dolédec (2004) in the Ardèche River, since they did not found any significant relationship of the trait Simpson diversity with hydraulics. In our study, all structural and functional metrics followed the same patterns and were positively correlated (Table 4). This correlation might be a result of a strong trait filtering by the local environmental constraints (Poff et al., 1997; Statzner et al., 2004; Bêche & Statzner, 2009), which seems to be very clear under different local hydraulic conditions in a eutrophied river as the Ebro, despite the recent decreases in phosphorous content (Ibáñez et al., 2008).

Table 4. Pearson correlation analysis of community metrics based on taxonomy and biological traits. All correlations are significant for $P<0.01$.

	Richness	Shannon diversity	Simpson diversity	Rao diversity
Trait richness	0.72	0.72	0.67	0.65
Trait Shannon diversity	0.79	0.81	0.78	0.73
Trait Simpson diversity	0.76	0.79	0.77	0.65
Trait Rao diversity	0.62	0.68	0.69	0.57

Hydraulic preferences of benthic macroinvertebrates

According to the literature, most of the macroinvertebrate hydraulic preferences obtained in this study agreed with the ecological classification into reophilic or limnephilic organisms (see Tachet et al., 2000; Merrit & Cummins, 2008; Wiederholm, 1983), although in the case of Chironomids the available information was scarce at genus or species level. In the Ebro, many of the hydraulic preferences of the studied

taxa were representative when analyzed also at family level, since a single species corresponded to a single family. For instance, this was the case of Oligochaeta, most of the Trichoptera and the Ephemeroptera, Odonata and Coleoptera. Thus, and as was expected, the Trichoptera *H. exocellata* showed hydraulic preferences for high water velocities, according to its feeding habits as a filter-feeder (Sagnes et al., 2008; Mérigoux & Dolédec, 2004). On the other hand, the species *E. tenellus* and *O. angustella* presented a significant hydraulic preference for low water velocities, while they did not show significant results in the study of Mérigoux & Dolédec (2004), probably due to the lower densities and seasonality of these taxa in the Ardèche River. *Ceraclea* spp., *P. pusilla*, *M. azurea* and *Hydroptila* sp. were not very abundant, thus it can be a reason why they did not show habitat marginality in this study. Several species of Ephemeroptera as *C. luctuosa*, *E. virgo* and *C. picteti* are considered limnephilic species (Tachet et al., 2000). However, in this study only *Caenis* presented hydraulic marginality for lentic areas while *E. virgo*, for instance, could also be present at higher water velocities. It has been reported that *E. virgo* can shift its hydraulic preferences along growth moving to areas with lower hydraulic stress as they increase in size (Sagnes et al., 2008). Moreover, even though this species has a synchronized life cycle, a deviation in mean size of the cohabiting specimens exists (see Cid et al., 2008); thus, different sizes might lead to a wider water velocity tolerance for this species. This was not the case of *Hydroptila* sp., since earlier larvae stages of Hydroptilidae showed the same hydraulic preferences as fully developed larvae. Most of Oligochaeta were found in slow flowing areas (most of them Naididae), as observed by Syrovatka et al. (2009), except for the lumbricid *E. tetraedra* and the Tubificidae SSC (without hair setae) which showed preference for areas with higher water velocity. However, many Naididae can be reophilic species and a wider range of hydraulic preferences of this group could be expected if a mesh smaller than 500 µm had been used in this study.

On the other hand, other studied taxa from the Ebro presented variability for the hydraulic preferences within the same family or within the same genus. In the case of Trichoptera, although the two *Ceraclea* species cohabiting in the Ebro did not present significant inter-genus variability for hydraulic preferences, *C. dissimilis* was present in samples with higher water velocities than *C. sobradieli*. In the case of ephemeropterans, the family Baetidae was represented by high abundances of the genus *Baetis* (*B. fuscatus and B. pavidus*) with clear preferences for habitats with high water velocity.

Many *Baetis* species are classified as reophilic species in other large Mediterranean rivers (Buffagni et al., 2010), however differences between species can also exist. In our case, the species *P. atrebatinus*, classified as *Baetis* few years ago (see Lugo-Ortiz et al., 1999), showed very different hydraulic preferences compared to *Baetis* species in the lower Ebro, occupying areas with medium to low water velocities. On the other hand, the genus *Cloeon* usually inhabits still or slowly moving waters (Edmunds et al., 1976; Tachet, 2000). Here, we observed that the species *C. dipterum* was only present in samples where water velocity was null while *C. simile* tolerated areas with low water velocity, although it has been reported before that both species cohabited in the same habitat (Sowa, 1980). The group of Chironomidae was present along the whole hydraulic gradient for the Ebro River, and different habitat preferences were also observed within the same subfamily, tribe or genus. This was the case of the Orthocladiinae subfamily. For instance, within the genus *Cricotopus*, *C. (C.) trifascia* and *C. (C.) albiforceps* distinctly inhabited fast flowing areas, while *C. (C.) bicinctus* inhabited medium-flow areas and the group of *C.* (*I.*) *sylvestris* had preferences for slow flowing areas. This niche separation demonstrates why Collier (1993) found a wide range of velocity optima when assessing the hydraulic preferences of *Cricotopus* spp. at genus level, not reflecting the interspecies variability. Within the Tanytarsini, though they were not very abundant, *Tanytarsus chinyensis* grp. was present in high velocity conditions, while *Tanytarsus pallidicornis* type preferred more lentic habitats, which agrees with the lotic and lentic character of this genus described by Merrit & Cummins (2008). The observed species-specific niche separation at local scale reflects the high diversity and adaptative radiation of the group of Chironomidae that can be influenced by hydraulic conditions and species interactions. Thus, this wide niche variability might be the reason why Chironomidae grouped as Orthocladiinae did not show habitat marginality in other studies of hydraulic preferences (Mérigoux & Dolédec, 2004). Therefore, this variability leads to the question whether taxonomy resolution might hide hydraulic preferences of several taxonomic groups. As mentioned by Dolédec et al. (2007), this study confirms that some macroinvertebrates which are usually being identified at genera or subfamily level (e.g. Orthocladiinae, Baetidae), and that may reflect variability for hydraulic preferences at a higher taxonomic resolution, should be identified at species level for a higher accuracy in the interpretation of results. Therefore, the taxonomy resolution in this case was fundamental to precisely determine the local mesohabitat macroinvertebrate assemblages. Furthermore, within the

Chironomidae, several species showed controversial hydraulic preferences according to the literature. For instance, the Orthocladiinae *S. semivirens* can inhabit both flowing and still water (Wiederholm, 1983). Compared to the Chironomid study of Syrovátka et al. (2009), *S. semivirens* in the Svratka River occurred at higher proportions in habitats with low current velocities, while in the Ebro it was very abundant in fast flowing areas. For this species, Syrovátka et al. (2008) had previously found opposite hydraulic preferences depending on the studied river. Thus, it means that *S. semivirens* is likely a generalist species which shifts its hydraulic preferences maybe due to competitive exclusion with other species present at the same ecological niche. In the Ebro, the presence of *Cladophora* sp.in high velocity areas can provide food and shelter for this species and, as mentioned above, can also change the near bed hydraulic conditions, increasing its potential habitat. Likewise, the genus *Rheotanytarsus* is considered reophilic (Wiederholm, 1983; Merrit & Cummins, 2008), but we found that it was present in medium flow areas. This can be due to the relatively low densities of this Tanytarsini, however there is a possibility that this filter-feeder could adapt to habitats of slow flowing water where the amount of suspended particles can be abundant. Moreover, *Rheotanytarsus* sp. is usually present in the lateral and bottom part of cobbles, a microhabitat where the hydraulic stress is reduced and favors the maintenance of its fragile tube case structure. Thus, in the context of the Ebro River, where constant flows are predominant, this taxon might have colonized areas with an intermediate flow.

In general, when performing analysis to quantify the hydraulic preferences of organisms (e.g. the OMI analysis) we should consider that different results can be obtained depending on the measured hydraulic range, the ecosystem characteristics at each study and the taxonomic resolution achieved for the community. However, even though considering those differences, similar patterns should be obtained for the most specialist species within different study approaches (Dolédec et al., 2007).

Macroinvertebrate trait response to the hydraulic gradient

The hydraulic conditions determined part of the macroinvertebrate functional composition at local scale, evidencing that the traits are a primary filter to determine the community composition as a result of an evolutionary process (Townsend & Hildrew, 1994). This is supported by Lamoroux et al. (2004) since many traits reflected an

adaptation to habitat conditions. However, some traits are difficult to relate to hydraulic characteristics since life history adaptations seem to be a strategy for long-term flow patterns (Lytle & Poff, 2004). Accordingly, life history, behavioral and morphological traits should be differently interpreted.

Morphological traits such as small body size have been predicted to increase with increasing flow, while intermediate and largest sizes should decrease (Statzner & Bêche, 2010). Several studies observed that the proportion of potential small invertebrates was greater in habitats with stressful hydraulic constraints (Lamoroux et al., 2004; De Crespin et al., 2002; Snook & Milner, 2002), in agreement with other studies concerning flow adaptations (see Statzner, 2008). In contrast, Mérigoux & Dolédec (2004) obtained the opposite results. Since this prediction can vary depending on the substratum coarseness (Statzner, 1981) and the developmental stage (e.g., instar) of the invertebrates (Sagnes et al., 2008), it can be difficult to interpret in terms of adaptation to flow, since many evolutionary and ecological processes might intervene (Statzner et al., 2004). In this study, we show also contradictory results since the smallest organisms (<0.5 cm, small taxa as Naididae species, *Micronecta* sp. or larvae of coleoptera as *Dryops* sp.) and the largest (>8 cm, only represented by the species *B. sowerbyi*) were present in slow-flowing areas, and only organisms with a maximal size of 2-4 cm were associated to areas with higher velocities. Other size categories did not show any significant relationship with flow. The weak association of flow with other size categories might be explained by the high proportion of organisms with a size of 0.5-2 cm across all the sampled area. Moreover, we should consider the lack of maximal size information on Chironomidae genus and species in the trait database used (Tachet et al., 2000). For example, small sized Orthocladiinae as *Thiennemanniella* sp. and *Synorthocladius* sp. (with a maximal size of 0.3 and 0.4 cm, respectively; see Wiederholm, 1983) were more abundant in fast flowing areas in this study, but were not taken into account since the traits only considered the Orthocladiinae as a group, including those living both in lentic and lotic environments. Therefore, within the trait analysis of the macroinvertebrate community, the lack of biological traits for genus or species of Chironomidae might hide some responses of traits to environmental factors due to the high proportion of this taxonomic group in the present study.

Because the costs for macroinvertebrates inhabiting fast flowing habitats are compensated by the food and oxygen availability (Hynes, 1970; Williams & Hynes,

1973; Peckarsky et al., 1990) their hydraulic preferences should be in accordance to feeding strategies, respiration and locomotion or substrate relation. As might be expected, the proportion of filter-feeders increased with higher hydraulic stress, while deposit feeders were abundant in slow-flowing habitats, according to other studies (Doisy et al., 2001; Lamoroux et al., 2004; Mérigoux & Dolédec, 2004). On the other hand, other feeding strategies (scrapers, absorbers, predator and parasites) can differ depending on the study in question. For instance, predators did not show any significant relationship with hydraulics in the present study, while others observed a positive (Rempel et al., 2000; Doisy et al., 2001) or negative correlation (Lamoroux et al., 2004). According to Statzner & Bêche (2010), organisms temporarily attached to the substrate (e.g. anal claws of *H. exocellata*) were favored in fast flowing areas as a result of morphological adaptation, while swimmers decreased, in agreement with Snook & Milner (2002) and with Horrigan & Baird (2008). The proportion of crawlers (e.g. *Baetis* spp.) presents a more variable response when comparing different studies (see Tomanova & Usseglio-Polatera, 2007; Horrigan & Baird, 2008) while burrowers (both epibenthic and endobenthic) are clearly typical from slow flowing areas (Lamoroux et al., 2004; Tomanova & Usseglio-Polatera, 2007; this study). Respiration by gills was predominant in stressful habitats, according to Horrigan & Baird (2008), though different results were obtained by Lamoroux et al. (2004). These differences in our study can be caused by the high relative proportions of *H. exocellata* having non mobile gills in comparison to *E. virgo* which inhabit areas of moderate velocity and its densities were much lower. The increase of tegument respiration in lentic areas in the present study is in agreement with Tomanova & Usseglio-Polatera (2007), while the opposite pattern predicted by Statzner & Bêche (2010) was also obtained by Horrigan & Baird (2008). For plastron and aerial respiration, which were positively correlated with slow flowing areas, the results were also very different depending on the study in question.

For those traits related with life history, our results suggest that organisms living in fast flowing areas do not present resistance forms or that eggs can be a strategy to adapt to disturbances, while cocoons were representative of slow-flowing areas. Also voltinism, reproduction and dispersal appeared to be linked to hydraulics. Most of these results were consistent with Lamoroux et al. (2004) except for ovoviviparity, which was favored in fast flowing areas, and dispersal, showing different significant patterns. However, some significant associations of life history traits with the local

environmental variables can be misinterpreted since many other filters at a larger spatial and temporal scale might intervene and many traits can be intercorrelated (Statzner et al., 2004; Lamoroux et al., 2004). For instance, organisms with more than one life cycle per year and with a short lifespan are favored in Mediterranean areas, independently of the habitat characteristics (Bonada et al., 2007). However, the latter authors found no significant differences for life cycle duration at different flow conditions, nor did we for different hydraulic conditions. Although floods in the Ebro are reduced in magnitude and frequency due to river regulation, natural floods used to occur in autumn and in spring and, therefore, life cycle should be synchronized with those flow events as a long-term survival strategy. Also other strategies related to life cycle as diapause stage or aerial dispersal might be related with life-history evolutionary processes (e.g. *E. virgo*). In the case of alien species, well established populations of *C. fluminea* in the lower Ebro can be favored by their ovoviviparous reproduction, long life cycle and more reproductive cycles per year (Statzner et al., 2008).

Concluding remarks

In the context of regulated rivers, within the expected hypotheses in response to flow alteration, those concerning invertebrate communities involve shifts in species richness, abundance and distribution (Poff et al., 2010), and the species presenting high habitat hydraulic marginality would be those more sensitive to hydrological alterations (Dolédec et al., 2007). Thus, the understanding of the hydraulic influence in the community assemblage and in its functional structure can have potential applications in the guiding management of the lower Ebro River. Moreover, as hydraulic habitat variables determining many macroinvertebrate traits at microhabitat level can predict reach-scale responses (Lamoroux et al., 2004), this functional approach can also be a useful base for ecological indicators, though there is a need to include the species biological traits of Chironomidae. In general, by delimiting the macroinvertebrate assemblages in function of the hydraulic conditions the different species-specific niche occupation was determined and different responses within the analyzed taxonomic groups were observed. For instance, co-ocurrence of species for the same hydraulic conditions existed (e.g. species of *Ceraclea*, *Baetis*, *Cloeon* or some *Cricotopus*). On the other hand, the niche separation into different hydraulic conditions for same genus

(e.g. within *C. trifascia* and *C. bicinctus*) was also present reflecting ecological processes related with species competitive exclusion. Because the macroinvertebrate assemblages were mainly defined by the different hydraulic conditions, many of the biological traits analyzed also were structured along this gradient, though some of them could reflect long term adaptations to flow disturbances or to species interactions. Overall, this study highlights the complexity of species assemblages and their functional structure at local scale, and demonstrates that the higher is the taxonomic resolution (either for taxa analysis and the trait database used), the more precise will be our understanding of their response according to the hydraulic conditions.

Acknowledgements

This study was funded by the Government of Catalonia (Agència Catalana de l'Aigua and Departament d'Innovació, Universitats i Empresa), the European Social Fund and the Government of Spain (Ministerio de Educación y Ciencia, research project CGL2006-01487, Plan Nacional I+D+I; Ministerio de Medioambiente y Medio Rural y Marino). We thank the Ebro Water Authority (Confederación Hidrográfica del Ebro) for the data on water discharge. We are very grateful to Sylvain Dolédec (traits database), Núria Bonada (*Ceraclea* species identification and statistics), Iraima Verkaik and Cesc Múrria (statistics), and David Mateu (sampling support). Also special thanks to Manuel Graça and Maria Joao Feio for their comments and support throughout the manuscript writing.

References

Bady, P., Dolédec, S., Fesl, C., Gayraud, S., Bacchi, M. & Scholl, F. (2005) Use of invertebrate traits for the biomonitoring of European large rivers: the effects of sampling effort on genus richness and functional diversity. *Freshwater Biology*, **50**, 159-173.

Batalla, R.J., Gómez, C.M. & Kondolf, G.M. (2004) Reservoir-induced hydrological changes in the Ebro River basin (NE Spain). *Journal of Hydrology*, 290, 117-136.

Batalla, R.J. & Vericat, D. (2009) Hydrological and sediment transport dynamics of flushing flows: implications for management in large Mediterranean rivers. *River Research and Applications*, **25**, 297-314.

Bêche, L.A. & Statzner, B. (2009) Richness gradients of stream invertebrates across the USA: taxonomy- and trait-based approaches. *Biodiversity and Conservation,* **18,** 3909-3930.

Blettler, M., Amsler, M., De Drago, I.E. & Marchese, M. (2008) Effects of stream hydraulics and other environmental variables on density of *Narapa bonnettoi* (Oligochaeta) in the Parana River System. *River Research and Applications*, **24**, 1124-1140.

Bonada, N., Rieradevall, M. & Prat, N. (2007) Macroinvertebrate community structure and biological traits related to flow permanence in a Mediterranean river network. *Hydrobiologia*, **589**, 91-106.

Brinkhurst, R.O. (1971) A guide for the identification of British aquatic Oligochaeta. *Scientific Publication of the Freshwater Biological Association* 22: 1–55.

Brooks, S.J., Langdon, P.G. & Heiri, O. (2007) The identification and use of Palaearctic Chironomidae larvae in palaeoecology, In: *Quaternary Research Association Technical Guide no. 10.* 276.

Buffagni, A., Erba, S. & Armanini, D.G. (2010) The lentic-lotic character of Mediterranean rivers and its importance to aquatic invertebrate communities. *Aquatic Sciences,* **72,** 45-60.

Champely, S. & Chessel, D. (2002) Measuring biological diversity using Euclidean metrics. *Environmental and Ecological Statistics,* **9**, 167-177.

Chevenet, F., Dolédec, S. & Chessel, D. (1994) A fuzzy coding approach for the analysis of long-term ecological data. *Freshwater Biology*, **31**, 295-309.

Cid, N., Ibáñez, C. & Prat, N. (2008) Life history and production of the burrowing mayfly *Ephoron virgo* (Olivier, 1791) (Ephemeroptera: Polymitarcyidae) in the lower Ebro River: A comparison after 18 years. *Aquatic Insects*, **30**, 163-178.

Collier, K.J. (1993) Flow preferences of larval Chironomidae (Diptera) in Tongariro River, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, **27**, 219-226.

De Crespin de Billy V. & Usseglio-Polatera P. (2002) Traits of brown trout prey in relation to habitat characteristics and benthic invertebrate communities. *Journal of Fish Biology*, **60**, 687–714.

Doisy, K.E. & Rabeni, C.F. (2001) Flow conditions, benthic food resources, and invertebrate community composition in a low-gradient stream in Missouri. *Journal of the North American Benthological Society*, **20**, 17-32.

Dolédec, S., Chessel, D., Ter Braak, C. J. F. & Champely, S. (1996) Matching species traits to environmental variables: a new three-table ordination method. *Environmental and Ecological Statistics*, **3**, 143–166.

Dolédec, S., Chessel, D. & Gimaret-Carpentier, C. (2000) Niche separation in community analysis: A new method. *Ecology*, **81**, 2914-2927.

Dolédec, S., Lamouroux, N., Fuchs, U. & Mérigoux, S. (2007) Modelling the hydraulic preferences of benthic macroinvertebrates in small European streams. *Freshwater Biology*, **52**, 145-164.

Dunbar, M.J., Pedersen, M.L., Cadman, D., Extence, C., Waddingham, J., Chadd, R. & Larsen, S.E. (2010) River discharge and local-scale physical habitat influence macroinvertebrate LIFE scores. *Freshwater Biology*, **55**, 226-242.

Dray, S. & P. Legendre (2008). Testing the link between species traits and environmental characetristics: the fourthcorner problem revisited. *Ecology*, **89**, 3400-3412.

Edmunds, G.F., Jensen, S.L. & Berner, L. (1976) *The mayflies of North and Central America*, University Minnesona press, Minneapolis.

Ferrarese, U. (1983). Chironomidi, 3 (Diptera, Chironomidae: Tanypodinae). In: S. Ruffo, ed., Guide per il riconoscimento delle specie animali delle acque interne italiane. CNR, AQ/1/204, 26, pp. 67.

Gore, J.A. (1978) Technique for predicting in-stream flow requirements of benthic macroinvertebrates. *Freshwater Biology*, **8**, 141-151.

Gore, J.A., Layzer, J.B. & Mead, J. (2001) Macroinvertebrate instream flow studies after 20 years: A role in stream management and restoration. *Regulated Rivers-Research & Management*, **17**, 527-542.

Hart, D.D. & Finelli, C.M. (1999) Physical-biological coupling in streams: The pervasive effects of flow on benthic organisms. *Annual Review of Ecology and Systematics*, **30**, 363-395.

Heiri,O., Eckrem, T. & Willassen, E. (2004) Larval head capsules of European Micropsectra, Paratanytarsus and Tanytarsus (Diptera: Chironomidae: Tanytarsini). http://www.bio.uu.nl/~palaeo/Chironomids/Tanytarsini/Heiri,%20Ekrem,%20Willassen%202004%20v.%201.0.pdf

Horrigan N. & Baird D.J. (2008) Trait patterns of aquatic insects across gradients of flow-related factors: a multivariate analysis of Canadian national data. *Canadian Journal of Fisheries and Aquatic Sciences*, **65**, 670–680.

Hynes, H.B.N. (1970) Ecology of stream insects. *Annual Review of Entomology*, **15**, 25-42.

Ibáñez, C. & Prat, N. (2003) The environmental impact of the Spanish National Hydrological Plan on the lower Ebro River and delta. *International Journal of Water Resources Development*, **19**, 485-500.

Ibañez, C., Prat, N., Duran, C., Pardos, M., Munné, A., Andreu, R., Caiola, N., Cid, N., Hampel, H., Sánchez, R. & Trobajo, R. (2008) Changes in dissolved nutrients in the lower Ebro River: Causes and consequences. *Limnetica*, **27**, 131-142.

Ihaka R, Gentleman R (1996) R: a language for data analysis and graphics. *Journal of Computational and Graphical Statistics*, **5**, 299–314.

James, A.B.W. & Suren, A.M. (2009) The response of invertebrates to a gradient of flow reduction - an instream channel study in a New Zealand lowland river. *Freshwater Biology*, **54**, 2225-2242.

Lamouroux, N., Dolédec, S. & Gayraud, S. (2004) Biological traits of stream macroinvertebrate communities: effects of microhabitat, reach, and basin filters. *Journal of the North American Benthological Society*, **23**, 449-466.

Lancaster, J. & Belyea, L.R. (2006) Defining the limits to local density: alternative views of abundance-environment relationships. *Freshwater Biology*, **51**, 783-796.

Lancaster, J. & Hildrew, A.G. (1993) Flow refugia and the microdistribution of lotic macroinvertebrates. *Journal of the North American Benthological Society*, **12**, 385-393.

Legendre, P., Galzin, R. & Harmelin-Vivien, M. (1997) Relating behavior to habitat: solutions to the fourthcorner problem. *Ecology*, **78**, 547–562.

Lugo-Ortiz, C.R., Mccafferty, W.P. & Waltz, R.D. (1999) Definition and reorganization of the genus *Pseudocloeon* (Ephemeroptera: Baetidae) with new species descriptions and combinations. *Transactions of the American Entomological Society*, **125**, 1-37.

Lytle, D.A. & Poff, N.L. (2004) Adaptation to natural flow regimes. *Trends in Ecology & Evolution*, **19**, 94-100.

Malard, F., Galassi, D., Lafont, M., Dolédec, S. & Ward, J.V. (2003) Longitudinal patterns of invertebrates in the hyporheic zone of a glacial river. *Freshwater Biology*, **48**, 1709-1725.

Malavoi, J.R. & Souchon, Y. (2002) Standardized description of streams and rivers channel geomorphic units: Qualitative description key and physical measurements. *Bulletin Francais de la Peche et de la Pisciculture*, 357-372.

Mérigoux, S. & Dolédec, S. (2004) Hydraulic requirements of stream communities: a case study on invertebrates. *Freshwater Biology*, **49**, 600-613.

Mérigoux, S., Lamouroux, N., Olivier, J.M. & Dolédec, S. (2009) Invertebrate hydraulic preferences and predicted impacts of changes in discharge in a large river. *Freshwater Biology*, **54**, 1343-1356.

Merritt, R.W., K.W. Cummins, and M.B. Berg, eds. (2008) An Introduction to the Aquatic Insects of North America. 4th Ed., Kendall/Hunt Publishing Co., Dubuque, Iowa.

Müller-Liebenau, I. (1969) Revision der europäischen Arten der Gattung Baetis Leach, 1815 (Insecta, Ephemeroptera). Gewäss Abwäss 28/29: 1–214.

Muñoz, I. & Prat, N. (1994) Macroinvertebrate community in the lower Ebro River (NE Spain). *Hydrobiologia*, 286, 65-78.

Múrria, C. (2009) Understanding the influence of environmental factors and past geological events on the genetic structure of aquatic insects using population and community approaches. PhD thesis, University of Barcelona.

Nocentini, A.M. (1985). Chironomidi, 4 (Diptera, Chironomidae: Chironominae, larve). In: S. Ruffo, ed., Guide per il riconoscimento delle specie animali delle acque interne italiane. CNR, AQ/1/233, 29, pp. 186.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., O'Hara, R.G., Simpson, G.L., Solymos, P., Stevens, H. & Wagner, H. (2010) Vegan: Community Ecology Package. R package version 1.17-0. http://CRAN.R-project.org/package=vegan

Peckarsky, B.L., Horn, S.C. & Statzner, B. (1990) Stonefly predation along a hydraulic gradient- a field- test of the harsh benign hypothesis. *Freshwater Biology*, **24**, 181-191.

Poff, N.L. (1997) Landscape filters and species traits: Towards mechanistic understanding and prediction in stream ecology. *Journal of the North American Benthological Society,* **16,** 391-409.

Poff, N.L., Olden, J.D., Vieira, N.K.M., Finn, D.S., Simmons, M.P. & Kondratieff, B.C. (2006) Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships. *Journal of the North American Benthological Society*, **25**, 730-755.

Poff, N.L., Richter, B.D., Arthington, A.H., Bunn, S.E., Naiman, R.J., Kendy, E., Acreman, M., Apse, C., Bledsoe, B.P., Freeman, M.C., Henriksen, J., Jacobson, R.B., Kennen, J.G., Merritt, D.M., O'keeffe, J.H., Olden, J.D., Rogers, K., Tharme, R.E. & Warner, A. (2010) The ecological limits of hydrologic alteration (ELOHA): a new framework for developing regional environmental flow standards. *Freshwater Biology*, **55**, 147-170.

Quinn, J.M. & Hickey, C.W. (1994) Hydraulic parameters and benthic invertebrate distributions in 2 gravel-bed New-Zealand rivers. *Freshwater Biology*, **32**, 489-500.

Rader, R.B. (1997) A functional classification of the drift: traits that influence invertebrate availability to salmonids. *Canadian Journal of Fisheries and Aquatic Sciences,* **54,** 1211-1234.

Rempel, L.L., Richardson, J.S. & Healey, M.C. (1999) Flow refugia for benthic macroinvertebrates during flooding of a large river. *Journal of the North American Benthological Society*, **18**, 34-48.

Rempel, L.L., Richardson, J.S. & Healey, M.C. (2000) Macroinvertebrate community structure along gradients of hydraulic and sedimentary conditions in a large gravel-bed river. *Freshwater Biology*, **45**, 57-73.

Rieradevall, M. & Brooks, S.J. (2001) An identification guide to subfossil Tanypodinae larvae (Insecta : Diptera : Chironomidae) based on cephalic setation. *Journal of Paleolimnology*, **25**, 81-99.

Rossaro, B. (1982). Chironomidi, 2 (Diptera, Chironomidae: Orthocladiinae). In: S. Ruffo, ed., Guide per il riconoscimento delle specie animali delle acque interne italiane. CNR, AQ/1/171, 16, pp. 80.

Ruse, L.P. (1994) Chironomid microdistribution in gravel of an English chalk river. *Freshwater Biology*, **32**, 533-551.

Sabater, S., Artigas, J., Duran, C., Pardos, M., A. M. Romaní, A. M., Tornes, E. & Ylla, I. (2008) Longitudinal development of chlorophyll and phytoplankton assemblages in a regulated large river (the Ebro River). *Science of the Total Environment*, **404**, 196-206.

Sagnes, P., Mérigoux, S. & Peru, N. (2008) Hydraulic habitat use with respect to body size of aquatic insect larvae: Case of six species from a French Mediterranean type stream. *Limnologica*, **38**, 23-33.

Schmid, P. E. (1993) A key to the larval Chironomidae and their instars from Austrian Danube region streams and rivers. Part 1: Diamesinae, Prodiamesinae and Orthocladiinae. Wasser und Abwasser Suppl. 3/93, 514.

Snook D.L. & Milner A.M. (2002) Biological traits of macroinvertebrates and hydraulic conditions in a glacier-fed catchment (French Pyréneés). *Archiv für Hydrobiologie*, **153**, 245–271.

Sowa, R. (1980) Taxonomy and ecology of European species of the Cloeon-simile Eaton group (Ephemeroptera, Baetidae). *Entomologica Scandinavica*, **11**, 249-258.

Statzner, B. (1981) The relation between hydraulic stress and micro-distribution of benthic macroinvertebrates in a lowland running water-system, the Schierenseebrooks (North Germany). *Archiv Fur Hydrobiologie*, **91**, 192-218.

Statzner, B., Gore, J.A. & Resh, V.H. (1988) Hydraulics stream ecology: observed patterns and potential applications. *Journal of the North American Benthological Society*, **7**, 307-360.

Statzner, B. & Higler, B. (1986) Stream hydraulics as major determinant of benthic invertebrate zonation patterns. *Freshwater Biology*, **16**, 127-139.

Statzner, B., Hildrew, A.G. & Resh, V.H. (2001) Species traits and environmental, constraints: Entomological research and the history of ecological theory. *Annual Review of Entomology*, **46**, 291-316.

Statzner, B., Dolédec, S. & Hugueny, B. (2004) Biological trait composition of European stream invertebrate communities: assessing the effects of various trait filter types. *Ecography,* **27,** 470-488.

Statzner, B. (2008) How Views about Flow Adaptations of Benthic Stream Invertebrates Changed over the Last Century. *International Review of Hydrobiology*, **93**, 593-605.

Statzner, B., Bonada, N. & Dolédec, S. (2008) Biological attributes discriminating invasive from native European stream macroinvertebrates. *Biological Invasions*, **10**, 517-530.

Statzner, B. & L. A. Bêche. (2010) Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? *Freshwater Biology*, **55**, 80-119.

Steinman, A.D. and G. A. Lamberti. (1996) Biomass and pigments of benthic algae. P. 297. In "Methods in Stream Ecology". Hauer, F.R. and G.A. Lamberti (eds). Academic Press, San Diego, CA.

Suren, A.M. & Jowett, I.G. (2006) Effects of floods versus low flows on invertebrates in a New Zealand gravel-bed river. *Freshwater Biology*, **51**, 2207-2227.

Syrovátka, V. & Brabec, K. (2008) The response of chironomid larvae to hydraulic conditions: *Synorthocladius semivirens* (Diptera: Chironomidae) in two different rivers. *Boletim do Museu Municipal do Funchal, Supplement*, **13**, 161–167.

Syrovatka, V., Schenkova, J. & Brabec, K. (2009) The distribution of chironomid larvae and oligochaetes within a stony-bottomed river stretch: the role of substrate and hydraulic characteristics. *Fundamental and Applied Limnology*, **174**, 43-62.

Tachet, H., Ricoux, P., Bournaud, M. & Usseglio- Polatera, P. (2000) Invertebrés d'eau douce: systématique, biologie, écologie., CNRS Editions, Paris.

Thioulouse, J., Chessel, D., Dolédec, S. & Olivier, J.M. (1997) ADE-4: A multivariate analysis and graphical display software. *Statistics and Computing*, **7**, 75-83.

Thuiller, W., Lavorel, S., Midgley, G., Lavergne, S. & Rebelo, T. (2004) Relating plant traits and species distributions along bioclimatic gradients for 88 Leucadendron taxa. *Ecology*, **85**, 1688-1699.

Tomanova, S. & Usseglio-Polatera, P. (2007) Patterns of benthic community traits in neotropical streams: relationship to mesoscale spatial variability. *Fundamental and Applied Limnology,* **170,** 243-255.

Townsend, C.R. & Hildrew, A.G. (1994) Species traits in relation to a habitat templet for river systems. *Freshwater Biology*, **31**, 265-275.

Vericat, D. & Batalla, R.J. (2006) Sediment transport in a large impounded river: The lower Ebro, NE Iberian Peninsula. *Geomorphology*, **79**, 72-92.

Vieira, R. (2000) Las larvas de los Tricópteros de Galicia (Insecta: Trichoptera), Univesidad de Santiago de Compostela, Santiago de Compostela. PhD Thesis.

Wiederholm, T. (1983) Chironomidae of the Holarctic region. In: Keys and diagnoses. Part 1 Larvae, *Entomologica Scandinavica Supplement* **19**, 1- 457.

Williams, N. E., and H. B. N. Hynes. (1973) Micro-distribution and feeding of the net-spinning cad-disflies (Trichoptera) of a Canadian stream. *Oikos*, **24**, 73-84.

.

SUPPORTING INFORMATION

SI 1. Macroinvertebrate community OMI analysis.

	axis 1	axis 2
Eigenvalues	0.55	0.08
%variability	75.77	11.31
% accumulated variability	75.77	87.08
variable coordinates		
D	0.17	-0.02
U 0.6	-0.43	-0.03
Fr	-0.42	-0.06
Re	-0.39	0.01
DO in sediment	0.03	-0.18
%macrophyte	-0.13	0.21
variable normed scores		
D	0.23	-0.06
U 0.6	-0.58	-0.12
Fr	-0.56	-0.22
Re	-0.52	0.05
DO in sediment	0.04	-0.62
%macrophyte	-0.17	0.74

SI 2. Results of the linear regression analysis for each diversity measure and the water velocity.

	coefficient	intercept	Multiple R^2	Adjusted R^2	F	p-value
Taxon richness	-0.1770	36.87	0.44	0.42	26.6	1.08E-05
Shannon diversity	-0.0063	3.52	0.43	0.41	25.7	1.38E-05
Simpson diversity	-0.0003	0.97	0.37	0.35	19.8	8.78E-05
Rao diversity	-0.0158	1.85	0.61	0.60	53.98	1.63E-08
Trait richness	-0.0578	58.69	0.33	0.31	16.5	0.000269
Trait Shannon diversity	-0.0008	3.71	0.52	0.51	37.3	6.19E-07
Trait Simpson diversity	-0.00002	0.97	0.43	0.42	25.9	1.30E-05
Trait Rao diversity	-0.0019	1.87	0.18	0.16	7.67	0.00901

SI 3. Density (ind/m^2) of the macroinvertebrate taxa and mean water velocity for each Surber sample in the lower Ebro River

Surber samples	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
Velocity (cm/s)	0	0	14.1	11.5	0	3.8	74.6	85.3	84.4	67.5	87	64.8	60.4	76.4	52.4	14.1	0	0	0	5.5	15.9	15	14.1	12.4	11.4	15	66.6	47	34.6	31.9	29.2	29.2	70.1	56.8	33.7	43.2
Cnidaria																																				
Hydra sp.	8	4	0	0	0	0	0	0	0	0	0	0	4	4	0	0	4	0	0	0	64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Turbellaria																																				
Dugesia sp.	116	72	336	20	92	28	80	280	268	288	116	100	172	44	136	88	32	68	72	16	52	84	24	76	184	68	52	20	40	272	732	144	108	116	288	180
Microturbellaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nemertea																																				
Prostoma graecense (Böhmig)	8	4	0	0	0	0	0	4	0	0	0	0	44	0	76	32	0	0	100	88	40	8	88	20	36	8	0	16	68	32	80	36	0	0	0	96
Nematoda																																				
Mermithidae	8	0	0	0	0	0	0	8	64	0	0	4	0	0	0	0	0	0	0	0	4	0	0	4	36	0	0	0	16	0	128	0	0	0	0	0
Oligochaeta																																				
Branchiura sowerbyi Beddard	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	104	4	0	0	32	0	0	0	0	0	0	0	0	0	0	0	0	0
Potamothrix bavaricus (Oschmann)	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0
Potamothrix hammoniensis (Michaelsen)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0
Tubificidae SSC	0	0	32	0	0	0	0	0	0	0	0	0	0	0	8	32	0	0	0	0	32	4	4	0	0	0	0	0	0	0	44	0	0	0	48	0
Lumbriculidae	0	0	0	0	0	0	4	0	0	0	0	0	4	0	0	0	8	0	0	0	0	0	0	0	4	0	0	0	0	0	0	4	0	0	12	0
Chaetogaster diaphanus (Gruithuisen)	0	0	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dero digitata (Müller)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0
Nais bretscheri Michaelsen	24	0	0	80	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	4	4	0	0	0	0	32	0	0	0	0	0	0	0	0	0
Nais cf. barbata	0	64	0	0	144	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nais cf. pardalis	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nais cf.bretscheri	0	64	80	0	496	152	0	0	0	0	0	0	0	0	0	128	0	112	0	0	64	0	0	48	0	0	0	32	16	0	4	0	0	0	0	0
Nais sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	0
Ophidonais serpentina (Müller)	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	36	0	16	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Slavina appendiculata (Udeken)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	96	0	0	0	32	0	0	0	0	16	0	0	0	8	0

Continued from SI 3. Density (ind/m^2) of the macroinvertebrate taxa and mean water velocity for each Surber sample in the lower Ebro River.

Surber samples	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
Velocity (cm/s)	0	0	14.1	11.5	0	3.8	74.6	85.3	84.4	67.5	87	64.8	60.4	76.4	52.4	14.1	0	0	0	5.5	15.9	15	14.1	12.4	11.4	15	66.6	47	34.6	31.9	29.2	29.2	70.1	56.8	33.7	43.2
Stylaria lacustris (Linnaeus)	764	500	20	0	0	80	0	0	0	0	0	0	4	0	0	452	360	12	36	12	316	136	8	32	264	32	16	0	72	32	20	16	0	0	32	0
Vejdovskyella intermedia (Bretscher)	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	4	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eiseniella tetraedra (Savigny)	0	0	0	0	0	0	0	8	0	0	0	0	0	0	12	0	0	0	16	0	0	0	0	0	0	0	0	4	0	0	8	0	0	0	20	0
Lumbricidae others	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0
Hirudinea																																				
Erpobdella sp.	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0
Helobdella stagnalis (L.)	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Piscicola geometra (L.)	0	0	4	0	0	12	0	0	0	0	0	0	0	0	0	0	8	0	0	4	20	0	12	0	0	8	4	0	4	0	16	4	0	0	0	0
Mollusca																																				
Corbicula fluminea (O.F. Müller)	24	40	4	0	0	8	64	4	100	0	16	4	76	0	228	24	20	12	108	552	104	80	228	64	76	36	28	100	64	252	96	96	20	16	40	272
Dreissema polymporpha (Pallas)	0	0	0	0	4	0	0	4	8	4	0	4	0	4	4	4	0	0	12	0	0	0	12	8	0	0	4	4	4	0	0	0	12	28	0	0
Ferrissia (Pettancylus)clessiniana (Jickeli)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0
Physella (Costatella) acuta (Draparnaud)	0	0	12	0	20	20	0	0	0	0	0	0	20	48	20	0	0	0	112	32	4	32	140	24	36	164	0	0	0	16	12	0	0	8	24	8
Potamopyrgus antipodarum (Smith)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0
Radix sp.	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	8	8	0	0	0	0	0	0	0	8	0	4	0	0
Theodoxus fluviatilis (L.)	4	0	0	68	76	8	64	16	108	44	44	104	20	20	8	4	0	4	116	8	48	116	460	124	44	28	464	268	36	8	76	332	44	152	92	20
Hydracarina	8	4	256	0	4	24	256	0	0	0	16	8	76	12	0	12	8	4	100	16	236	12	32	68	436	260	20	80	36	104	184	48	32	0	128	0
Microcrustacea																																				
Caridean larvae	0	168	0	0	0	0	0	0	0	0	0	0	0	0	0	0	508	20	0	16	160	132	64	64	32	128	0	0	0	0	0	0	0	0	0	0
Cladocera	4	288	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	0	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Copepoda	4	160	0	0	148	44	0	0	0	0	0	0	0	0	0	32	0	48	0	16	0	0	0	0	0	0	0	64	0	0	0	0	0	128	32	0
Ostracoda	72	732	0	0	84	148	0	0	0	0	0	0	8	0	64	268	104	20	296	344	96	96	108	0	288	68	0	72	32	4	120	16	0	0	32	0
Crustacea																																				
Atyaephyra desmarestii (Millet)	0	0	0	0	0	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Continued from SI 3. Density (ind/m^2) of the macroinvertebrate taxa and mean water velocity for each Surber sample in the lower Ebro River.

Surber samples	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
Velocity (cm/s)	0	0	14.1	11.5	0	3.8	74.6	85.3	84.4	67.5	87	64.8	60.4	76.4	52.4	14.1	0	0	0	5.5	15.9	15	14.1	12.4	11.4	15	66.6	47	34.6	31.9	29.2	29.2	70.1	56.8	33.7	43.2
Corophium sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
Echinogammarus longisetosus Pinkster	28	12	2872	244	752	176	7648	1816	11264	4508	4176	3708	1556	532	2520	132	88	240	404	20	84	296	96	76	128	416	1968	872	92	504	1160	1532	1548	2980	1660	968
Proasellus meridianus (Racovitza)	0	0	0	0	0	4	0	0	0	0	0	0	0	0	4	4	0	44	0	0	4	8	4	36	0	0	0	0	0	72	0	0	0	0	40	32
Ephemeroptera																																				
Baetis fuscatus (L.)	40	8	6308	72	4	460	3372	14820	3760	1084	1068	2196	1184	808	3408	84	0	12	16	24	144	184	24	76	1148	240	264	472	304	400	720	504	636	916	1076	2008
Baetis pavidus Grandi	8	0	920	32	0	104	28	360	916	116	104	416	32	56	1564	8	0	0	0	0	248	0	0	0	196	8	80	84	32	16	196	180	92	568	320	1128
Caenis luctuosa (Burmeister)	64	300	48	0	52	20	0	36	12	0	0	0	32	0	148	688	284	212	480	308	996	196	192	68	388	268	24	72	88	308	20	8	0	4	52	60
Choroterpes picteti (Eaton)	88	88	8	4	20	12	312	104	12	0	0	0	228	12	8	12	12	16	60	4	8	20	8	20	12	28	0	8	24	40	4	4	4	0	20	0
Cloeon dipterum (L.)	0	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cloeon simile Eaton	0	0	0	0	48	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0
Ephoron virgo (Olivier)	28	4	0	0	0	0	64	28	60	0	0	4	120	12	56	0	0	4	8	0	68	24	0	4	0	20	0	12	16	20	0	12	4	0	0	0
Pseudocloeon atrebatinus (Eaton)	8	0	0	0	0	44	0	0	0	0	0	0	0	0	0	32	0	8	0	4	444	0	4	48	128	0	0	0	0	0	64	0	0	0	0	16
Odonata																																				
Coenagrionidae immature	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0
Platycnemis sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	4	0	0	12	8	16	0	0	0	0	0	4	16	0	0	36	0
Zigoptera immature	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	32	0	0	0	0	0	0	0	0	0	0	0	0
Heteroptera																																				
Gerris sp.	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrometra sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	4
Micronecta sp.	4	224	56	0	68	16	0	0	0	0	0	0	16	0	0	16	12	28	0	16	4	0	0	0	0	0	8	0	0	0	0	0	0	8	0	0
Naucoris maculatus Fabricius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
Collembola	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	0	0

Continued from SI 3. Density (ind/m^2) of the macroinvertebrate taxa and mean water velocity for each Surber sample in the lower Ebro River.

Surber samples	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
Velocity (cm/s)	0	0	14.1	11.5	0	3.8	74.6	85.3	84.4	67.5	87	64.8	60.4	76.4	52.4	14.1	0	0	0	5.5	15.9	15	14.1	12.4	11.4	15	66.6	47	34.6	31.9	29.2	29.2	70.1	56.8	33.7	43.2
Neuroptera-Plannipennes																																				
Sysira sp.	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coleoptera																																				
Aulonogyrus sp.	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dryops sp.	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	4	0	0	0	0	0	32	0	0	0	0	0	0	8	0	0	0	0
Hydaticus sp.	4	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Laccophilus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	12	4	8	8	4	4	0	0	0	0	0	0	4	0	0	0	0	4
Pomatinus substriatus (P.H. Müller)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Potamophilus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0
Trichoptera																																				
Ceraclea dissimilis (Stephens)	0	0	0	0	4	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	16	0	0	0	0	0
Ceraclea sobradieli (Navás)	0	0	0	0	4	0	0	0	4	0	0	0	0	0	0	0	0	0	4	0	0	0	12	0	4	0	0	4	4	0	4	12	0	0	0	0
Ecnomus tenellus (Rambur)	8	4	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	12	16	8	8	4	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsyche exocellata Dufour	4	4	44	12	4	12	132	288	184	172	148	100	76	172	228	12	8	8	4	0	92	4	4	20	8	8	300	88	48	16	440	148	108	432	220	744
Hydroptila sp.	44	84	116	124	8	12	76	124	32	8	36	12	60	32	12	208	68	56	16	0	24	68	36	12	48	44	52	100	48	184	8	44	292	8	4	192
Hydroptilidae stage 1-4	4	0	52	0	4	0	192	0	64	0	0	4	40	52	0	32	36	16	32	16	96	32	16	128	64	64	48	0	16	64	64	8	32	0	32	0
Mystacides azurea (L.)	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0
Ortotrichia angustella (McLachlan)	16	4	0	4	0	8	0	0	0	0	0	0	0	0	0	0	12	8	36	4	68	0	8	4	4	0	0	0	0	0	0	0	4	0	0	0
Psycomia pusilla (Fabricius)	0	12	0	0	0	8	0	0	0	4	0	0	4	8	4	16	0	4	16	0	20	20	4	4	16	112	112	24	88	100	128	0	32	0	0	4
Diptera-Chironomidae																																				
Tanypodinae																																				
Ablabesmyia longistyla Fittkau	4	28	8	16	4	0	0	0	0	0	0	0	0	0	0	48	4	12	92	0	88	32	32	8	32	40	4	0	48	0	0	0	0	0	0	16
Thienemannimyia sp.	0	0	4	4	0	0	0	0	0	0	0	0	0	0	0	4	0	0	4	0	0	0	0	0	4	32	4	4	0	24	4	4	0	0	8	0
Procladius sp.	8	12	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Continued from SI 3. Density (ind/m^2) of the macroinvertebrate taxa and mean water velocity for each Surber sample in the lower Ebro River.

Surber samples	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
Velocity (cm/s)	0	0	14.1	11.5	0	3.8	74.6	85.3	84.4	67.5	87	64.8	60.4	76.4	52.4	14.1	0	0	0	5.5	15.9	15	14.1	12.4	11.4	15	66.6	47	34.6	31.9	29.2	29.2	70.1	56.8	33.7	43.2
Diamesinae																																				
Potthastia gaedii (Meigen)	0	33	0	0	0	0	0	0	0	0	0	0	0	0	0	74	0	0	20	0	0	0	34	20	43	65	0	0	0	0	0	0	0	0	0	0
Orthocladiinae																																				
Cricotopus (C.) festivellus(Kieffer)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Cricotopus (I.) sylvestris grp.	352	952	2264	565	1707	539	18	20	90	0	0	0	178	150	8	670	1825	780	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cricotopus (C.) albiforceps(Kieffer)	0	0	0	0	0	0	282	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	78	0	0	146	140	54	168	0	0	55	0	0	0
Cricotopus (C.)bicinctus (Meigen)	232	218	5000	605	969	413	581	41	311	1355	741	862	356	226	50	573	333	41	552	20	989	781	316	784	1881	770	524	593	664	214	6522	1821	55	4589	1858	340
Cricotopus (C.) trifascia Edwards	0	0	0	0	0	0	282	426	306	0	0	230	0	226	8	0	0	0	0	0	0	0	0	78	0	0	0	36	0	0	0	0	218	98	0	0
Cricotopus vierriensis grp.	352	218	431	1766	969	248	4235	847	269	1435	2247	1323	1368	1504	657	199	498	350	106	16	342	251	167	20	589	1090	1222	598	2245	3754	282	41	6990	292	238	549
Eukiefferiella gracei (Edwards)	0	0	0	0	0	0	0	0	0	262	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eukiefferiella minor-fitkawi	12	0	0	4	72	36	76	100	0	312	364	128	36	211	196	32	64	0	0	0	0	0	0	0	0	4	64	28	0	0	0	0	452	202	0	16
Orthocladius (O.) obumbratus Johansen	0	0	0	0	0	0	18	0	0	0	0	0	121	0	0	397	664	741	126	0	45	0	0	0	0	0	0	0	278	137	0	0	0	0	0	0
Orthocladius sp. D	0	0	0	0	0	0	0	0	0	0	0	86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Parametriocnemus stylatus (Kieffer)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0	0	0	0	0	0	0	0	0	0	0	0	0
Paratrichocladius rufiventris (Meigen)	0	0	0	0	0	165	0	0	0	0	0	0	0	0	0	0	0	0	259	0	0	333	0	0	0	0	0	0	54	0	0	0	164	0	63	0
Rheocricotopus (Ps.) chalybeatus (Edwards)	0	0	694	320	0	0	845	214	132	0	0	238	81	75	960	0	0	0	0	0	0	0	0	0	0	0	0	36	278	672	1591	61	331	155	238	351
Rheocricotopus (Ps.) fuscipes (Kieffer)	4	0	100	0	16	8	0	68	76	4	0	0	12	4	72	4	0	0	0	0	0	35	4	0	87	16	4	8	82	32	12	4	16	32	104	8
Synorthocladius semivirens (Kieffer)	0	0	340	192	0	32	512	32	128	68	48	132	100	44	320	100	0	0	76	0	476	116	28	20	420	360	20	96	204	556	196	4	144	0	128	72
Thienemanniella flaviforceps (Kieffer)	0	0	304	0	0	8	0	0	64	0	0	64	0	4	512	32	0	0	32	20	808	0	68	240	856	64	16	0	0	0	400	32	0	128	776	960
Chironominae																																				
Chironomini																																				
Cryptochironomus sp.	0	20	0	0	0	0	4	0	0	0	0	0	4	0	0	4	4	0	8	0	4	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
Dicrotendipes nervosus grp.	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Harnischia sp.	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	8	0	0	32	0	20	0	8	16	0	0	0	0	4	0	0	4	0	0	0	0

Continued from SI 3. Density (ind/m^2) of the macroinvertebrate taxa and mean water velocity for each Surber sample in the lower Ebro River.

Surber samples	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
Velocity (cm/s)	0	0	14.1	11.5	0	3.8	74.6	85.3	84.4	67.5	87	64.8	60.4	76.4	52.4	14.1	0	0	0	5.5	15.9	15	14.1	12.4	11.4	15	66.6	47	34.6	31.9	29.2	29.2	70.1	56.8	33.7	43.2
Polypedilum nubeculosum grp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0
Polypedilum (P.) nubifer (Skuse)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0
Polypedilum (T.) scalaenum grp.	12	36	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Polypedilum type A (sensu Brooks et al., 2007)	4	16	0	0	20	8	0	0	0	0	0	32	0	0	0	8	0	52	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0
Stenochironomus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tanytarsini																																				
Cladotanytarsus sp.	0	32	16	0	0	0	0	0	0	0	0	0	0	0	0	32	0	0	0	0	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rheotanytarsus sp.	44	20	164	0	0	8	0	0	0	0	0	0	0	0	4	8	0	4	0	0	4	0	4	0	68	32	0	0	0	0	0	0	0	0	0	48
Tanytarsus chinyensis grp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32
Tanytarsus forma larval 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tanytarsus pallidicornis type (sensu Heiri et al., 2001)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	40	44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tanytarsus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Virgatanytarsus sp.	8	0	4	0	0	0	0	0	0	0	0	0	0	0	4	116	40	12	0	0	4	0	0	0	4	32	4	0	0	0	0	0	0	0	0	8
Diptera-Others																																				
Atrichops crassipes Meigen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Berdeniella sp.	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ceratopogoninae sp1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hemerodromia sp.	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	40
Hexatoma sp.	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Simulium erytrocephalum (De Geer)	0	0	40	0	0	0	0	0	8	0	4	8	0	0	452	4	0	0	44	0	160	8	16	168	776	0	0	0	0	4	1020	0	44	176	660	164

SI 4. Table of relative abundance of traits per sample.

Surber		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
Maximal size	<0.25 cm	0.02	0.06	0.05	0.02	0.07	0.05	0.00	0.01	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.03	0.03	0.05	0.00	0.03	0.02	0.00	0.00	0.01	0.00	0.00	0.03	0.01	0.01	0.00	0.00	0.00	0.00	0.03	0.01	0.00
	>0.25–0.5 cm	0.15	0.19	0.16	0.20	0.16	0.14	0.08	0.11	0.09	0.08	0.14	0.08	0.09	0.09	0.11	0.15	0.18	0.21	0.18	0.15	0.17	0.14	0.14	0.14	0.18	0.12	0.15	0.13	0.11	0.15	0.11	0.10	0.17	0.10	0.10	0.18
	>0.5–1 cm	0.37	0.36	0.37	0.42	0.37	0.36	0.38	0.37	0.33	0.38	0.35	0.36	0.35	0.39	0.35	0.35	0.31	0.37	0.31	0.25	0.36	0.37	0.33	0.37	0.35	0.38	0.37	0.37	0.35	0.40	0.30	0.30	0.38	0.37	0.35	0.41
	>1–2 cm	0.31	0.24	0.21	0.19	0.20	0.23	0.23	0.26	0.28	0.21	0.18	0.27	0.31	0.30	0.26	0.25	0.22	0.20	0.26	0.29	0.27	0.32	0.26	0.29	0.31	0.33	0.20	0.23	0.33	0.27	0.32	0.31	0.17	0.19	0.27	0.22
	>2–4 cm	0.10	0.10	0.15	0.17	0.18	0.18	0.23	0.19	0.23	0.31	0.29	0.26	0.17	0.21	0.17	0.13	0.16	0.15	0.13	0.13	0.10	0.11	0.15	0.13	0.10	0.12	0.19	0.17	0.14	0.14	0.15	0.19	0.22	0.25	0.15	0.14
	>4–8 cm	0.04	0.04	0.07	0.00	0.02	0.04	0.06	0.06	0.08	0.01	0.04	0.03	0.05	0.01	0.11	0.07	0.08	0.03	0.09	0.14	0.07	0.06	0.11	0.05	0.05	0.05	0.06	0.10	0.06	0.05	0.11	0.08	0.05	0.06	0.12	0.05
	>8 cm	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.00	0.02	0.01	0.00	0.00	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00
Life cycle duration	<1 year	0.62	0.67	0.62	0.68	0.68	0.62	0.65	0.62	0.58	0.62	0.59	0.65	0.63	0.71	0.60	0.55	0.60	0.63	0.59	0.56	0.63	0.57	0.62	0.59	0.63	0.72	0.64	0.59	0.63	0.63	0.53	0.54	0.67	0.65	0.50	0.65
	>1 year	0.38	0.33	0.38	0.32	0.32	0.38	0.35	0.38	0.42	0.38	0.41	0.35	0.37	0.29	0.40	0.45	0.40	0.37	0.41	0.44	0.37	0.43	0.38	0.41	0.37	0.28	0.36	0.41	0.37	0.37	0.47	0.46	0.33	0.35	0.50	0.35
Potential no. reproductive cycles per year	<1	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.00	0.01	0.02	0.00
	1	0.50	0.38	0.42	0.44	0.43	0.40	0.48	0.52	0.53	0.45	0.46	0.49	0.50	0.54	0.52	0.37	0.34	0.37	0.53	0.54	0.50	0.48	0.55	0.51	0.51	0.49	0.42	0.49	0.52	0.50	0.51	0.54	0.47	0.47	0.43	0.52
	>1	0.50	0.62	0.58	0.56	0.56	0.60	0.52	0.48	0.47	0.55	0.53	0.51	0.50	0.46	0.48	0.63	0.66	0.63	0.47	0.44	0.50	0.52	0.43	0.47	0.48	0.51	0.58	0.51	0.48	0.50	0.47	0.44	0.53	0.52	0.55	0.48
Aquatic stages	Egg	0.32	0.29	0.32	0.32	0.34	0.33	0.36	0.38	0.38	0.34	0.34	0.34	0.37	0.37	0.36	0.31	0.30	0.30	0.34	0.39	0.33	0.36	0.34	0.35	0.33	0.33	0.34	0.36	0.35	0.36	0.36	0.36	0.34	0.36	0.35	0.30
	Larva	0.37	0.38	0.37	0.34	0.35	0.35	0.36	0.36	0.34	0.33	0.34	0.35	0.34	0.33	0.34	0.36	0.38	0.38	0.33	0.31	0.36	0.33	0.31	0.32	0.35	0.35	0.33	0.33	0.34	0.34	0.32	0.33	0.34	0.32	0.34	0.35
	Pupa	0.17	0.19	0.15	0.20	0.13	0.13	0.14	0.11	0.11	0.16	0.16	0.17	0.11	0.15	0.13	0.16	0.17	0.17	0.15	0.07	0.16	0.12	0.16	0.15	0.16	0.17	0.15	0.13	0.15	0.13	0.13	0.12	0.19	0.14	0.11	0.19
	Adult	0.15	0.14	0.16	0.15	0.18	0.19	0.14	0.15	0.17	0.16	0.17	0.15	0.18	0.16	0.16	0.17	0.15	0.15	0.18	0.23	0.16	0.19	0.19	0.18	0.16	0.16	0.18	0.17	0.16	0.17	0.19	0.19	0.13	0.18	0.19	0.16
Reproduction type	Ovoviviparity	0.09	0.09	0.12	0.13	0.17	0.16	0.20	0.15	0.21	0.23	0.22	0.22	0.16	0.16	0.19	0.12	0.14	0.17	0.14	0.12	0.09	0.13	0.14	0.16	0.09	0.13	0.20	0.17	0.12	0.18	0.10	0.14	0.20	0.24	0.16	0.18
	Isolated eggs, free	0.11	0.10	0.06	0.03	0.07	0.08	0.09	0.09	0.09	0.00	0.02	0.06	0.10	0.05	0.10	0.09	0.09	0.10	0.08	0.07	0.11	0.12	0.04	0.08	0.06	0.09	0.06	0.09	0.10	0.10	0.06	0.08	0.05	0.04	0.07	0.04
	Isolated eggs, cemented	0.18	0.19	0.14	0.18	0.22	0.22	0.20	0.19	0.16	0.13	0.12	0.12	0.19	0.14	0.12	0.14	0.17	0.17	0.15	0.22	0.17	0.20	0.17	0.17	0.13	0.17	0.19	0.18	0.17	0.15	0.14	0.16	0.12	0.15	0.15	0.10
	Clutches, cemented or fixed	0.35	0.33	0.42	0.41	0.29	0.33	0.36	0.44	0.41	0.47	0.45	0.42	0.39	0.50	0.45	0.35	0.31	0.31	0.43	0.44	0.40	0.35	0.44	0.38	0.42	0.38	0.38	0.42	0.41	0.41	0.50	0.39	0.47	0.41	0.36	0.44
	Clutches, free	0.09	0.12	0.09	0.09	0.09	0.05	0.04	0.02	0.03	0.04	0.04	0.07	0.05	0.03	0.05	0.11	0.11	0.12	0.09	0.02	0.09	0.05	0.09	0.08	0.09	0.11	0.06	0.04	0.08	0.06	0.05	0.06	0.04	0.05	0.05	0.08

Continued from SI 4. Table of relative abundance of traits per sample.

Surber		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
	Clutches, in vegetation	0.02	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.02	0.00	0.00	0.03	0.02	0.05	0.01	0.00	0.00	0.00	0.00	0.02	0.04	0.00	0.00	0.04	0.01
	Clutches, terrestrial	0.05	0.05	0.05	0.07	0.07	0.05	0.06	0.04	0.05	0.07	0.10	0.07	0.04	0.06	0.05	0.05	0.05	0.04	0.06	0.05	0.05	0.04	0.05	0.06	0.08	0.05	0.04	0.04	0.04	0.05	0.05	0.06	0.07	0.07	0.05	0.11
	Asexual reproduction	0.11	0.11	0.10	0.09	0.08	0.11	0.05	0.06	0.05	0.06	0.05	0.04	0.08	0.06	0.04	0.12	0.14	0.10	0.05	0.06	0.10	0.10	0.04	0.07	0.07	0.06	0.07	0.05	0.07	0.06	0.08	0.07	0.04	0.04	0.12	0.03
Dispersal	Aquatic passive	0.37	0.41	0.41	0.42	0.45	0.47	0.42	0.40	0.44	0.42	0.41	0.42	0.42	0.41	0.42	0.46	0.47	0.45	0.43	0.47	0.42	0.46	0.44	0.43	0.38	0.41	0.46	0.45	0.39	0.41	0.44	0.39	0.39	0.45	0.52	0.38
	Aquatic active	0.32	0.30	0.29	0.29	0.31	0.30	0.29	0.32	0.30	0.33	0.31	0.30	0.33	0.31	0.31	0.28	0.26	0.26	0.30	0.33	0.29	0.30	0.29	0.31	0.31	0.30	0.30	0.30	0.33	0.33	0.31	0.34	0.30	0.31	0.26	0.32
	Aerial passive	0.15	0.16	0.13	0.14	0.11	0.11	0.12	0.12	0.12	0.10	0.11	0.13	0.11	0.11	0.12	0.13	0.15	0.16	0.13	0.08	0.14	0.11	0.13	0.13	0.15	0.14	0.11	0.11	0.13	0.13	0.11	0.09	0.15	0.11	0.09	0.13
	Aerial active	0.16	0.13	0.16	0.15	0.13	0.12	0.17	0.16	0.15	0.15	0.16	0.15	0.14	0.17	0.15	0.13	0.11	0.14	0.15	0.12	0.14	0.14	0.14	0.14	0.17	0.15	0.13	0.15	0.15	0.14	0.14	0.18	0.16	0.13	0.13	0.17
Resistance form	Eggs, statoblasts	0.13	0.10	0.11	0.08	0.07	0.09	0.21	0.20	0.18	0.08	0.09	0.12	0.17	0.15	0.18	0.08	0.06	0.10	0.11	0.08	0.12	0.14	0.07	0.12	0.11	0.12	0.06	0.13	0.13	0.15	0.11	0.10	0.13	0.11	0.12	0.10
	Cocoons	0.20	0.20	0.16	0.15	0.16	0.24	0.06	0.12	0.06	0.09	0.10	0.06	0.15	0.05	0.08	0.24	0.23	0.17	0.14	0.16	0.17	0.19	0.15	0.13	0.12	0.15	0.15	0.11	0.17	0.11	0.18	0.15	0.05	0.05	0.18	0.08
	Cells against desiccation	0.01	0.01	0.00	0.00	0.00	0.01	0.02	0.01	0.02	0.00	0.01	0.01	0.01	0.00	0.02	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.02
	Diapause or dormancy	0.07	0.11	0.11	0.10	0.23	0.12	0.08	0.05	0.10	0.09	0.10	0.12	0.11	0.10	0.12	0.10	0.08	0.14	0.12	0.17	0.11	0.12	0.18	0.18	0.15	0.12	0.16	0.10	0.08	0.10	0.12	0.15	0.10	0.20	0.16	0.15
	None	0.59	0.57	0.61	0.67	0.54	0.55	0.64	0.63	0.65	0.75	0.70	0.69	0.55	0.70	0.60	0.57	0.61	0.58	0.61	0.56	0.58	0.54	0.59	0.56	0.61	0.60	0.62	0.64	0.61	0.62	0.58	0.58	0.70	0.63	0.53	0.66
Respiration	Tegument	0.63	0.65	0.57	0.57	0.53	0.59	0.43	0.50	0.45	0.48	0.46	0.45	0.55	0.53	0.50	0.63	0.66	0.56	0.56	0.58	0.63	0.58	0.59	0.57	0.60	0.60	0.54	0.52	0.60	0.54	0.62	0.51	0.49	0.45	0.55	0.50
	Gill	0.33	0.31	0.35	0.43	0.42	0.35	0.57	0.50	0.54	0.52	0.50	0.50	0.43	0.47	0.47	0.34	0.32	0.39	0.39	0.37	0.32	0.39	0.38	0.40	0.34	0.39	0.44	0.48	0.40	0.45	0.34	0.46	0.48	0.49	0.42	0.41
	Plastron	0.01	0.02	0.02	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.01	0.01	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.01
	Spiracle (aerial)	0.03	0.02	0.07	0.00	0.03	0.05	0.00	0.00	0.01	0.00	0.03	0.05	0.01	0.00	0.03	0.02	0.01	0.03	0.04	0.03	0.04	0.03	0.02	0.04	0.06	0.02	0.01	0.00	0.00	0.01	0.04	0.02	0.03	0.05	0.03	0.09
Locomotion and substratum relation	Flier	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01
	Surface swimmer	0.02	0.01	0.02	0.01	0.02	0.03	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.01	0.02
	Full water swimmer	0.21	0.22	0.21	0.17	0.21	0.23	0.14	0.16	0.14	0.17	0.16	0.16	0.18	0.12	0.16	0.22	0.19	0.19	0.18	0.21	0.18	0.18	0.16	0.17	0.18	0.18	0.18	0.16	0.21	0.18	0.17	0.19	0.13	0.18	0.13	0.18
	Crawler	0.40	0.39	0.46	0.49	0.52	0.41	0.51	0.49	0.49	0.53	0.51	0.47	0.47	0.54	0.45	0.38	0.39	0.43	0.42	0.46	0.37	0.43	0.49	0.46	0.46	0.47	0.46	0.44	0.42	0.48	0.43	0.48	0.48	0.49	0.45	0.47
	Burrower (epibenthic)	0.14	0.14	0.08	0.07	0.07	0.08	0.14	0.12	0.14	0.02	0.08	0.11	0.13	0.07	0.14	0.14	0.09	0.13	0.16	0.19	0.14	0.12	0.12	0.10	0.09	0.10	0.08	0.16	0.12	0.13	0.10	0.11	0.08	0.05	0.10	0.09
	Interstitial (endobenthic)	0.08	0.10	0.09	0.10	0.08	0.09	0.08	0.08	0.06	0.07	0.07	0.07	0.09	0.06	0.09	0.13	0.17	0.11	0.08	0.05	0.09	0.14	0.07	0.09	0.10	0.11	0.07	0.08	0.08	0.09	0.10	0.08	0.06	0.06	0.17	0.09
	Temporarily attached	0.13	0.12	0.12	0.15	0.07	0.13	0.12	0.11	0.14	0.16	0.16	0.16	0.11	0.17	0.14	0.11	0.14	0.11	0.12	0.06	0.19	0.10	0.13	0.14	0.14	0.11	0.16	0.12	0.13	0.10	0.18	0.11	0.20	0.16	0.13	0.14
	Permanently attached	0.01	0.02	0.01	0.01	0.03	0.02	0.00	0.02	0.02	0.03	0.00	0.02	0.00	0.03	0.01	0.02	0.00	0.01	0.02	0.00	0.01	0.01	0.02	0.03	0.00	0.01	0.03	0.03	0.03	0.01	0.01	0.00	0.04	0.03	0.00	0.00

Continued from SI 4. Table of relative abundance of traits per sample.

Surber		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Food	Fine sediment+microorg.	0.02	0.03	0.03	0.02	0.03	0.02	0.01	0.02	0.01	0.00	0.00	0.00	0.01	0.00	0.02	0.03	0.03	0.03	0.02	0.02	0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.03	0.01	0.01	0.02	0.01	0.00	0.00	0.03	0.01
	Detritus < 1 mm	0.26	0.27	0.23	0.18	0.21	0.21	0.27	0.26	0.26	0.15	0.16	0.21	0.25	0.20	0.30	0.29	0.29	0.27	0.26	0.18	0.26	0.22	0.22	0.23	0.23	0.23	0.18	0.26	0.24	0.23	0.21	0.21	0.23	0.20	0.25	0.20
	Plant detritus > 1 mm	0.06	0.05	0.09	0.10	0.10	0.11	0.11	0.10	0.10	0.13	0.13	0.11	0.09	0.11	0.10	0.07	0.06	0.10	0.08	0.08	0.07	0.09	0.08	0.09	0.09	0.09	0.10	0.09	0.07	0.12	0.07	0.11	0.09	0.11	0.10	0.11
	Living microphytes	0.25	0.29	0.26	0.29	0.26	0.28	0.28	0.26	0.24	0.30	0.28	0.26	0.25	0.29	0.25	0.28	0.27	0.26	0.25	0.23	0.24	0.31	0.23	0.26	0.27	0.31	0.31	0.27	0.28	0.28	0.27	0.26	0.29	0.28	0.29	0.24
	Living macrophytes	0.10	0.10	0.09	0.14	0.12	0.10	0.10	0.09	0.08	0.10	0.10	0.10	0.10	0.12	0.08	0.08	0.11	0.11	0.10	0.08	0.09	0.09	0.11	0.09	0.09	0.10	0.11	0.10	0.08	0.10	0.07	0.08	0.13	0.10	0.07	0.09
	Dead animal > 1 mm	0.02	0.02	0.03	0.04	0.04	0.03	0.04	0.04	0.04	0.05	0.05	0.04	0.04	0.04	0.04	0.03	0.02	0.03	0.04	0.06	0.03	0.04	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.03	0.04	0.03	0.05	0.03	0.05
	Living microinvertebrates	0.11	0.13	0.10	0.09	0.10	0.11	0.10	0.08	0.10	0.12	0.12	0.11	0.11	0.12	0.09	0.09	0.10	0.09	0.10	0.13	0.13	0.10	0.12	0.12	0.09	0.07	0.12	0.10	0.09	0.08	0.10	0.10	0.12	0.14	0.10	0.11
	Living macroinvertebrates	0.18	0.11	0.14	0.14	0.15	0.10	0.10	0.16	0.17	0.17	0.15	0.17	0.15	0.11	0.12	0.12	0.08	0.11	0.15	0.19	0.14	0.13	0.16	0.16	0.17	0.13	0.10	0.11	0.17	0.14	0.21	0.16	0.11	0.11	0.12	0.19
	Vertebrates	0.00	0.00	0.02	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.03	0.00	0.00	0.02	0.03	0.00	0.03	0.00	0.00	0.02	0.02	0.00	0.02	0.00	0.03	0.02	0.00	0.00	0.00	0.00
Feeding habits	Absorber	0.00	0.01	0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.02	0.00	0.02	0.02	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.00	0.00	0.03	0.00
	Deposit feeder	0.22	0.25	0.20	0.16	0.20	0.21	0.16	0.20	0.14	0.09	0.08	0.10	0.18	0.12	0.18	0.27	0.28	0.24	0.20	0.18	0.19	0.20	0.16	0.15	0.17	0.20	0.15	0.20	0.18	0.16	0.16	0.14	0.10	0.09	0.22	0.11
	Shredder	0.15	0.13	0.19	0.18	0.22	0.20	0.21	0.17	0.19	0.22	0.22	0.20	0.16	0.17	0.16	0.14	0.15	0.22	0.15	0.16	0.13	0.17	0.16	0.16	0.12	0.15	0.18	0.16	0.12	0.19	0.12	0.18	0.16	0.18	0.16	0.18
	Scraper	0.21	0.23	0.26	0.31	0.29	0.29	0.25	0.23	0.22	0.31	0.29	0.28	0.24	0.34	0.23	0.23	0.18	0.21	0.24	0.24	0.21	0.28	0.24	0.26	0.28	0.30	0.30	0.24	0.24	0.26	0.24	0.25	0.27	0.31	0.26	0.23
	Filter feeder	0.14	0.15	0.11	0.08	0.07	0.10	0.21	0.17	0.24	0.17	0.19	0.21	0.18	0.20	0.25	0.12	0.12	0.12	0.17	0.13	0.19	0.16	0.16	0.19	0.14	0.13	0.18	0.20	0.18	0.18	0.17	0.15	0.27	0.25	0.16	0.20
	Piercer	0.08	0.07	0.09	0.09	0.05	0.09	0.04	0.04	0.02	0.03	0.04	0.02	0.07	0.03	0.02	0.05	0.10	0.08	0.06	0.09	0.08	0.04	0.06	0.04	0.05	0.05	0.05	0.03	0.04	0.03	0.03	0.03	0.07	0.03	0.01	0.06
	Predator	0.16	0.14	0.11	0.15	0.12	0.09	0.09	0.13	0.10	0.16	0.16	0.13	0.14	0.12	0.13	0.15	0.10	0.10	0.15	0.17	0.15	0.13	0.18	0.15	0.16	0.14	0.11	0.13	0.17	0.16	0.18	0.20	0.10	0.11	0.14	0.21
	Parasite	0.04	0.03	0.03	0.03	0.04	0.03	0.03	0.05	0.08	0.03	0.03	0.06	0.02	0.03	0.02	0.02	0.04	0.02	0.02	0.02	0.04	0.01	0.04	0.04	0.06	0.04	0.03	0.02	0.07	0.02	0.08	0.03	0.03	0.02	0.02	0.02

SI 5. Total density (ind/m^2) and occurrence of the macroinvertebrate taxa per sample unit in the lower Ebro River. See table SI3 for the authority of taxonomic nomenclature of species.

	Total Density	% density	Occurrence
Cnidaria			
Hydra sp.	88	0.03	6
Turbellaria			
Dugesia sp.	4864	1.77	36
Microturbellaria	20	0.01	1
Nemertea			
Prostoma graecense	884	0.32	20
Nematoda			
Mermithidae	272	0.10	9
Oligochaeta			
Branchiura sowerbyi	156	0.06	6
Potamothrix bavaricus	24	0.01	3
Potamothrix hammoniensis	24	0.01	2
Tubificidae SSC	204	0.07	8
Lumbriculidae	36	0.01	6
Chaetogaster diaphanus	16	0.01	1
Dero digitata	4	0.00	1
Nais bretscheri	160	0.06	6
Nais cf. Barbata	208	0.08	2
Nais cf. pardalis	8	0.00	1
Nais cf.bretscheri	1196	0.43	11
Nais sp.	32	0.01	1
Ophidonais serpentina	60	0.02	4
Slavina appendiculata	152	0.06	4
Stylaria lacustris	3216	1.17	22
Vejdovskyella intermedia	40	0.01	3
Eiseniella tetraedra	68	0.02	6
Lumbricidae others	4	0.00	1
Hirudinea			
Erpobdella sp.	8	0.00	2
Helobdella stagnalis	16	0.01	1
Piscicola geometra	96	0.03	11
Mollusca			
Corbicula fluminea	2856	1.04	32
Dreissema polymporpha	120	0.04	16
Theodoxus fluviatilis	3028	1.10	33
Physella (Costatella) acuta	752	0.27	19
Radix sp.	40	0.01	6
Ferrissia(Pettancylus) clessiniana	20	0.01	2
Potamopyrgus antipodarum	4	0.00	1
Hydracarina	2480	0.90	29
Microcrustacea			
Caridean larvae	1292	0.47	10

Continued from SI 5. Total density (ind/m^2) and occurrence of the macroinvertebrate taxa per sample unit in the lower Ebro River.

	Total Density	% density	Occurrence
Cladocera	356	0.13	4
Copepoda	676	0.25	10
Ostracoda	3072	1.12	22
Crustacea			
Atyaephyra desmarestii	32	0.01	1
Corophium orientale	4	0.00	1
Echinogammarus longisetosus	57076	20.75	36
Proasellus meridianus	252	0.09	11
Ephemeroptera			
Baetis fuscatus	47844	17.39	35
Baetis pavidus	7812	2.84	27
Cloeon dipterum	20	0.01	2
Cloeon simile	68	0.02	3
Pseudocloeon atrebatinus	800	0.29	11
Caenis luctuosa	5428	1.97	29
Choroterpes picteti	1200	0.44	30
Ephoron virgo	568	0.21	20
Odonata			
Coenagrionidae immature	8	0.00	1
Gomphus sp.	4	0.00	1
Platycnemis sp.	100	0.04	8
Zigoptera immature	36	0.01	2
Heteroptera			
Gerris sp.	4	0.00	1
Hydrometra sp.	8	0.00	2
Micronecta sp.	476	0.17	13
Naucoris maculatus	4	0.00	1
Collembola	56	0.02	3
Neuroptera-Plannipennes			
Sysira sp.	8	0.00	1
Coleoptera			
Aulonogyrus sp.	20	0.01	2
Dryops sp.	48	0.02	4
Hydaticus sp.	12	0.00	2
Laccophilus sp.	52	0.02	9
Pomatinus substriatus	4	0.00	1
Potamophilus sp.	4	0.00	1
Trichoptera			
Ceraclea dissimilis	28	0.01	4
Ceraclea sobradieli	52	0.02	9
Ecnomus tenellus	76	0.03	8
Hydropsyche exocellata	4292	1.56	35

Continued from SI 5. Total density (ind/m^2) and occurrence of the macroinvertebrate taxa per sample unit in the lower Ebro River.

	Total Density	% density	Occurrence
Hydroptila sp.	2292	0.83	35
Hydroptilidae stage 1-4	1208	0.44	26
Mystacides azurea	28	0.01	4
Ortotrichia angustella	180	0.07	13
Psycomia pusilla	740	0.27	22
Diptera-Chironomidae			
Tanypodinae			
Ablabesmyia longistyla	516	0.19	18
Procladius sp.	24	0.01	3
Thienemannimyia sp.	100	0.04	12
Diamesinae			
Potthastia gaedii	289	0.11	7
Orthocladiinae			
Cricotopus (C.) festivellus	4	0.00	1
Cricotopus (I.) sylvestris grp.	10117	3.68	15
Cricotopus (C.) albiforceps	923	0.34	7
Cricotopus (C.) bicinctus	36177	13.15	36
Cricotopus (C.) trifascia	1910	0.69	10
Cricotopus (C.) vierriensis grp.	37707	13.71	36
Eukieferiella gracei	262	0.10	1
Eukieferiella minor-fitkawi	2409	0.88	20
Orthocladius (O.) obumbratus	2527	0.92	9
Orthocladius sp. D	86	0.03	1
Parametriocnemus stylatus	38	0.01	1
Paratrichocladius rufiventris	1038	0.38	6
Rheocricotopus (Psilocrisotopus) chalybeatus	7273	2.64	18
Rheocricotopus (Ps.) fuscipes	812	0.30	25
Synorthocladius semivirens	4964	1.80	29
Thienemanniella flaviforceps	5388	1.96	20
Chironominae			
Chironomini			
Cryptochironomus sp.	52	0.02	8
Dicrotendipes nervosus grp.	16	0.01	3
Harnischia sp.	100	0.04	8
Polypedilum (P.) nubifer	8	0.00	2
Polypedilum (T.) scalaenum grp.	52	0.02	3
Polypedilum nubeculosum grp.	12	0.00	1
Polypedilum type A (sensu Brooks et al., 2007)	148	0.05	8
Stenochironomus sp.	4	0.00	1
Tanytarsini			
Cladotanytarsus sp.	112	0.04	4
Rheotanytarsus sp.	408	0.15	12

Continued from SI 5. Total density (ind/m^2) and occurrence of the macroinvertebrate taxa per sample unit in the lower Ebro River.

	Total Density	**% density**	**Occurrence**
Tanytarsus chinyensis grp.	64	0.02	2
Tanytarsus forma larval 1	32	0.01	1
Tanytarsus pallidicornis type (sensu Heiri et al., 2001)	84	0.03	2
Tanytarsus sp.	20	0.01	1
Virgatanytarsus sp.	236	0.09	11
Diptera-Others			
Hemerodromia sp.	48	0.02	3
Ceratopogoninae sp1	4	0.00	1
Simulium erytrocephalum	3756	1.37	18
Atrichops crassipes	4	0.00	1
Hexatoma sp.	4	0.00	1
Berdeniella sp.	4	0.00	1

SI 6. Environmental variables measured at each Surber sample. DO: dissolved oxygen in sediment.

Sample	Depth (cm)	Velocity (cm/s)	Fr	Re	DO (mg/l)	Macrophytes (%)
1	80	0	0	0	7.97	0
2	80	0	0	0	9.57	0
3	30	14.1	0.08	42300	8.34	25
4	68	11.5	0.04	78200	11.78	0
5	37	0	0	0	9.61	0
6	78	3.8	0.01	29640	9.54	0
7	43	74.6	0.36	320780	9.69	0
8	32	85.3	0.48	272960	9.54	0
9	37	84.4	0.44	312280	9.92	50
10	37	67.5	0.35	249750	9.99	0
11	85	87	0.3	739500	10.12	0
12	65	64.8	0.26	421200	10.07	25
13	100	60.4	0.19	604000	11.48	0
14	54	76.4	0.33	412560	10.72	0
15	34	52.4	0.29	178160	9.23	50
16	73	14.1	0.05	102930	11.48	0
17	67	0	0	0	8.82	0
18	50	0	0	0	9.29	0
19	74	0	0	0	9.4	0
20	80	5.5	0.02	44000	9.65	0
21	60	15.9	0.07	95400	10.27	25
22	78	15	0.05	117000	11.37	0
23	84	14.1	0.05	118440	11.37	0
24	74	12.4	0.05	91760	11.37	25
25	74	11.4	0.04	84360	9.75	25
26	76	15	0.05	114000	11.02	0
27	60	66.6	0.27	399600	9.38	0
28	67	47	0.18	314900	9.16	0
29	70	34.6	0.13	242200	9.65	0
30	82	31.9	0.11	261580	9.61	0
31	50	29.2	0.13	146000	4.52	100
32	83	29.2	0.1	242360	10.63	25
33	50	70.1	0.32	350500	11.5	0
34	48	56.8	0.26	272640	9.86	0
35	76	33.7	0.12	256120	4.55	0
36	29	43.2	0.26	125280	11.15	0

SI 7. Diversity indices calculated for each Surber sample.

Sample	Richness	Shannon diversity	Simpson diversity	Rao diversity	Trait richness	Trait Shannon diversity	Trait Simpson diversity	Trait Rao diversity
1	41	3.610	0.970	2.208	61	3.718	0.970	1.944
2	35	3.457	0.966	2.344	58	3.714	0.970	1.836
3	32	3.354	0.962	1.322	60	3.727	0.970	1.839
4	18	2.786	0.933	0.851	52	3.647	0.968	1.527
5	29	3.239	0.957	2.536	56	3.690	0.970	1.753
6	35	3.480	0.967	1.424	57	3.694	0.969	1.837
7	23	3.062	0.950	0.792	54	3.647	0.968	1.772
8	24	3.074	0.949	0.830	55	3.658	0.969	1.780
9	25	3.140	0.954	0.843	55	3.664	0.969	1.903
10	14	2.517	0.913	0.218	50	3.592	0.967	1.501
11	16	2.660	0.924	0.854	56	3.653	0.968	1.645
12	22	2.963	0.943	0.655	53	3.649	0.969	1.672
13	32	3.371	0.963	0.932	58	3.686	0.970	1.938
14	24	3.086	0.951	0.671	51	3.600	0.967	1.604
15	32	3.339	0.961	0.817	55	3.678	0.970	1.777
16	49	3.787	0.975	1.915	61	3.719	0.970	1.777
17	32	3.368	0.962	2.709	58	3.710	0.970	1.906
18	33	3.408	0.964	1.800	57	3.709	0.970	1.806
19	40	3.613	0.971	1.943	59	3.717	0.970	1.922
20	30	3.318	0.960	2.326	62	3.720	0.970	2.152
21	47	3.755	0.975	1.691	59	3.723	0.970	1.932
22	31	3.364	0.963	1.192	57	3.691	0.970	1.826
23	42	3.649	0.972	1.647	60	3.717	0.970	1.950
24	38	3.572	0.970	1.646	58	3.715	0.971	1.849
25	38	3.547	0.969	1.416	60	3.718	0.970	1.828
26	34	3.459	0.967	1.027	54	3.664	0.969	1.723
27	27	3.191	0.955	0.886	57	3.689	0.969	1.809
28	30	3.323	0.962	1.080	55	3.677	0.969	1.754
29	33	3.431	0.966	1.023	55	3.680	0.969	1.846
30	29	3.292	0.961	1.026	54	3.661	0.969	1.754
31	36	3.475	0.966	1.074	60	3.699	0.970	1.888
32	31	3.320	0.960	1.196	60	3.715	0.970	1.999
33	25	3.138	0.954	0.844	53	3.643	0.968	1.667
34	23	3.043	0.949	1.085	57	3.665	0.969	1.725
35	31	3.373	0.964	1.091	59	3.709	0.970	1.827
36	29	3.276	0.959	1.426	56	3.684	0.969	1.748

Chapter 3

Life history and production of the burrowing mayfly *Ephoron virgo* (Olivier, 1791) (Ephemeroptera: Polymitarcyidae) in the lower Ebro River: a comparison after 18 years.

Cid, N., Ibánez, C., and Prat, N. 2008.

***Aquatic insects* 30 (3):163–178.**

Life history and production of the burrowing mayfly *Ephoron virgo* (Olivier, 1791) (Ephemeroptera: Polymitarcyidae) in the lower Ebro River: A comparison after 18 years

Núria Cid[a]*, Carles Ibáñez[a] & Narcís Prat[b]

[a] *Aquatic Ecosystems Unit (UEA), Institute of Research and Technology, Food and Agriculture (IRTA), Poble Nou road, Km 5.5, P.O.Box 200, E-43540 Sant Carles de la Ràpita, Catalonia, Spain.*
[b] *Department of Ecology, Faculty of Biology, University of Barcelona, Diagonal 645, E-08028 Barcelona, Catalonia, Spain.*

Keywords: *Ephoron virgo*, life history, secondary production, temperature, Ebro River.

Corresponding author. Email: nuria.cid@irta.es

Abstract

Life history of the burrowing mayfly *Ephoron virgo* (Olivier, 1791) (Ephemeroptera: Polymitarcyidae) was studied during spring and summer 2005 in the lower Ebro River (Catalonia) and compared to a previous study performed in 1987 (Ibáñez et al. 1991). The results showed an advancement of *Ephoron virgo* life cycle and an increase of production estimates. In 2005 larval development reached the maximum size one month earlier than in 1987, and adult emergence peak began 3 weeks earlier. Comparing adult sex ratios (F:M), there was a major presence of females in 2005 (1:4), while the opposite was observed in 1987 (2:1). Secondary production was higher in 2005 than in 1987, obtaining 950 mg dry weight m^{-2} ·$year^{-1}$ with the increment summation method and 1080 mg dry weight m^{-2} $year^{-1}$ using the removal summation method. Higher water temperatures were measured for the entire 2005 larval growth period, which were related to higher air temperatures. Therefore, that temperature increment was likely the main cause of changes observed in *Ephoron virgo* life cycle.

Introduction

Ephoron virgo (Olivier, 1791) (Ephemeroptera: Polymitarcyidae) is a burrowing mayfly that inhabits many European and North African rivers, producing massive swarms in some of them. The filter-feeding larvae of this species construct U-shaped cavities in the riverbed in order to feed on suspended particles from the water currents produced by the movement of the tracheal gills. Its life cycle has been described earlier (Kureck & Fontes 1996; Ibañez 1991). It is characterised by a diapause egg stage persisting during autumn and winter which is broken in mid April when the larvae hatch. The growing period begins at this time and lasts until August, when male subimagines and females emerge. During a very short time males and females mate, and females oviposite. *E. virgo* has been absent for decades in most of the polluted rivers in Central Europe like the Rhine. Its return in the 1990s due to an improvement of water quality makes this species a good bioindicator of ecological quality (Kureck & Fontes 1996) of rivers. In the lower Ebro River *Ephoron virgo* usually inhabits areas of running water (Escosa et al. 1989) with substrates made up of gravel, cobbles with sand and fine sediments in the interstices, a habitat similar to those was observed in the Ardèche River (Mérigoux & Dolédec 2004). The species is not present in estuarine areas of the Ebro River with high salinity affected by the salt wedge (Muñoz & Prat 1993). At ecosystem level *E. virgo* is an important prey for fish and birds and a key species in the organic matter processing (Van der Geest et al. 2000). It enhances aerobic microbial activity by oxygenating river sediment in the microhabitat created in the burrows (Stief et al. 2004).

The study of secondary production gives information about alterations in river function, reflecting changes in organism activities (Benke 1993), and is an estimation of the ecological yield of the studied population. Some secondary production studies are based on spatial and temporal variation of the species or communities (Benke et al. 1984; Snyder et al.1991; Buffagni & Comin 2000; González et al. 2003a); others have compared production in regulated rivers before and after a reservoir (Rader & Ward 1989) or the effect of floods in unstable rivers (Scrimgeour 1991). How competition influences production rates on species in the same trophic level (González et al. 2003b) or how predators could decrease production (Iversen & Thorup 1987; Lugthgart & Wallace 1992) was also described. But most of the studies are performed in small streams and only a few are known from large rivers. Therefore our study is a

contribution to the understanding and estimation of *E. virgo* secondary production in lowland rivers.

During the last years *E. virgo* adult mass emergences seemed to be less abundant in the lower Ebro than in the 1980s and the adults were not present in areas far from the river (up to 40 km) were they used to fly in the past. This suggested that larval densities along the river could have declined, and consequently a lower production should be expected. As global warming has likely caused air temperature to increase during the last 3 decades in the studied area, and significant effects on phenology of terrestrial species of birds, insects and plants have been reported (Gordo et al. 2005), changes in *E. virgo* phenology may be attributed to climatic changes. Effectively, water temperature has also increased in the lower Ebro River basically due to higher air temperatures, lower discharges and the presence of reservoirs and a nuclear power station (Prats et al. in press). Thus, knowing that *E. virgo* egg hatching is dependent on temperature, changes in its life cycle may be expected.

The main objective of this study was to compare results of the life history and secondary production in 2005 with those found 18 years ago (Ibáñez et al. 1991). We studied (1) larval densities, (2) developmental patterns, (3) life cycle period and (4) adult emergences in order to estimate *E. virgo* population dynamics and secondary production in the present ecological context.

Materials and methods

Study Site

The Ebro River is one of the four most important river discharging to the Mediterranean Sea. It is located in NE Spain and has a drainage basin of 85 550 km^2 with a length of 928 km. The lower part of the river (100 km from the river mouth) is regulated by two main hydropower dams (Mequinença and Riba-Roja) constructed in the late sixties. A nuclear power plant was built in 1984 in Ascó, 15 km downstream of the dams. The sampling zone was located 40 km from river mouth (Fig. 1), upstream the city of Tortosa, the same site studied in 1987 (Ibañez et al. 1991) and not influenced by the salt wedge. The riverbed substrate consisted of sand, gravel and cobbles.

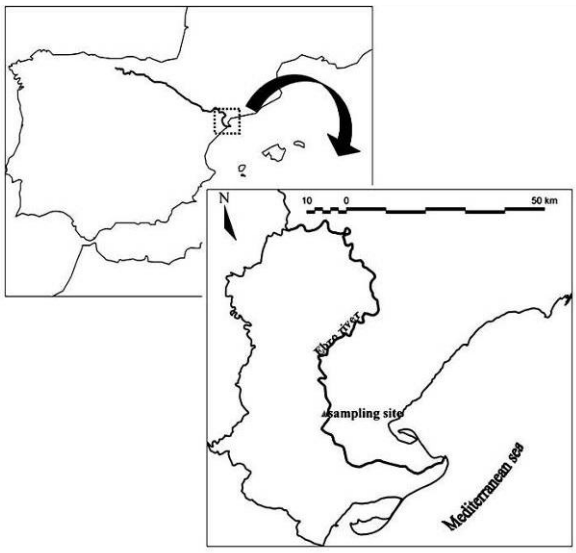


Figure 1. Study area in the lower Ebro river, located 40 km upstream of river mouth.

In this area the river is 200 m wide with a low water flow. Its maximum depth is about 2.5 m. Due to the deep main channel it was not possible to sample across the width of the river as it was done in 1987, but numerous areas with suitable depth and substrate were available. Mean daily discharge and temperature from the hydrological year 2004-2005 are shown in Fig. 2. During the sampling period, monthly mean discharge were 332.46, 217.21, 184.55, 145.44 and 108.18 $m^3 \cdot s^{-1}$ for April, May, June, July and August, respectively, while the annual mean discharge in Tortosa is 396 $m^3 \cdot s^{-1}$ (1960-2005, Data from the Water Authority: Confederación Hidrográfica del Ebro, C.H.E.).

Sampling of larvae and laboratory methods

The sampling period began at the end of May 2005. Samples were collected every 2 weeks until August, when samples were taken every week. In order to obtain a quantitative estimate of larval densities we took 8 random benthic samples in the studied area with a 0.25 m^2 Surber sampler (250 µm mesh net). The river bed was disturbed to a depth of 10 cm and contents were washed into the Surber net and deposited into a plate to identify and sort *E. virgo* larvae. Due to the difficulty to find larvae in August, extra kick samples were taken to obtain more accurate body parameters during this period. Larvae were placed in 5 ml plastic vials and remained there for 6 months in 70% EtOH. Due to the small size of the larvae in the initial samplings, we sorted the benthic samples *in vivo* at the laboratory under a dissecting

microscope to avoid field counting errors. Larvae were measured at the laboratory taking individual digital pictures with a Colorview Soft Imaging System camera attached to a Nikon SMZ800 stereoscopic microscope using the image analysis software Analysis SYS GMBH. We took three body measures to describe growth patterns: (a) body length (BL) (mm), distance between the end of the abdomen and the frontal process; (b) head width (HW) (mm), distance from eye to eye; and (c) wing pad length (WPL) (mm), length of the developing wing bud on the mesothorax (mm). The last body measure was not possible to be taken in all individuals caught before early July.

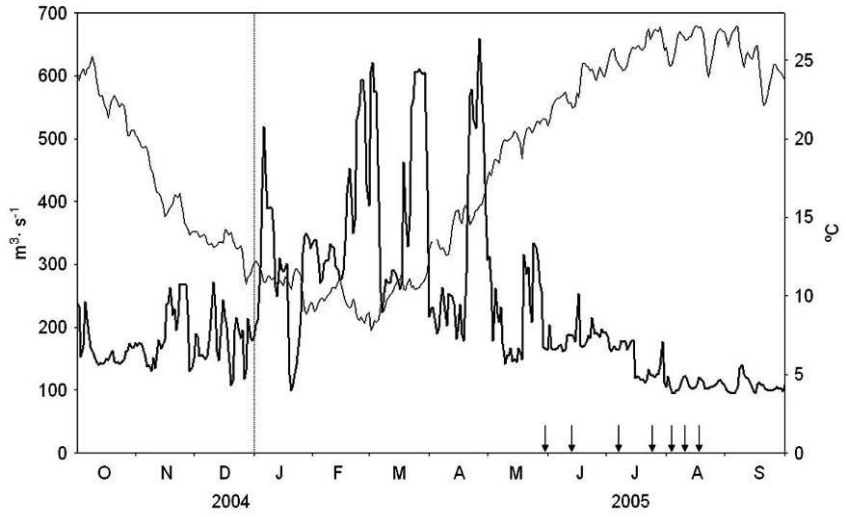


Figure 2. Mean daily discharge (thick lines) and mean daily temperature (light lines) during the hydrological year 2004-2005. Arrows show sampling dates of larvae.

To determine the relationship between BL and dry weight (DW) all larvae were dried at 60ºC for 24h, placed to a desiccator for 1 h and weighted on an electronic balance BEL Ultramark 205 with a resolution of 0.00001g. Larvae weighting was done in groups of individuals corresponding to each Surber sample. The individual mass was obtained from the individual mean weight of all Surber samples. In order to determine the effects of preservation on the biomass living larvae were collected from the same site. Half of the larvae were measured and dried with the same method cited above but individually weighted with an electronic balance Sartorius BP211D with a resolution of 0.00001 g. The remaining larvae were stored 6 months in ethanol 70% and also measured and dried individually. Effects of preservation with EtOH resulted in a weight loss around 50%,

depending on the size class (Fig. 3). As studies in 1987 were performed with preserved larvae these data were used to correct data from that year. Therefore, we applied a correction factor for each size class to avoid an underestimation of biomass and secondary production calculations.

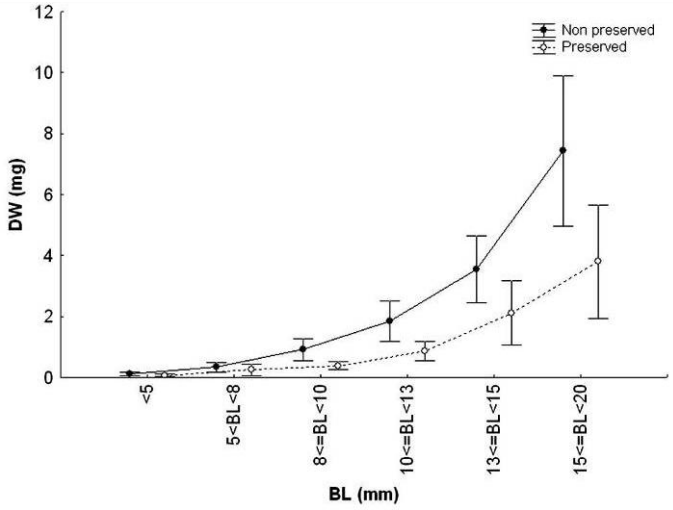


Figure 3. Relationship between body length (BL) and dry weight (DW) according to size classes from non preserved and preserved larvae of *Ephoron virgo*.

Secondary production calculation

The production was calculated for a period of 82 days (from 29th May to 19th August 2005) using 2 methods that may be applied to species with an unequivocal univoltine cycle: increment summation (IS) (Benke 1984; Rigler & Downing 1984) and removal summation (RS) (Waters & Crawford 1973).

Temperatures and degree days

Degree days (DD) were calculated for each month during the hydrological year and for the whole larval period (April-August). Due to the lack of experimental data on the hatching temperature of *E. virgo* and knowing that egg hatch is in April, we assumed a threshold temperature of 14.5 ºC, corresponding to the mean monthly temperature of water in April 2005 and in April 1987. Only for 2005 data were available at fifteen minute intervals for water temperatures (Xerta automatic station of the Water Authority:

C.H.E.), so we could obtain an accurate daily mean record for that period. DD estimates were calculated using the following equation: DD=Σ (T-To) (Southwood 1978) where T= daily mean temperature and To= threshold temperature for development.

It was not possible to strictly compare DD from 2005 and 1987 because in 1987 we only had one temperature measurement per month. However, the monthly mean from weekly measures in 2005 and the measures in 1987 (C.H.E.) were used to compare both years. Applying the above equation but modifying the total number of DD for both years we obtained: DD=Σ (Tm-To) d where Tm= monthly mean and d= number of days in the month. To support this approximation we used daily mean air temperatures (Ebro Observatory) to make correlation analyses with daily mean water temperatures (available from 1996 to 2005).

Adult collection

Adults were sampled twice a week in August 2005, with a total of 8 different sampling days during the emergence period. The sampling time began at dusk, when the emergence occurs, and ended 50 minutes later. Due to its positive phototropism adults were trapped using car lights situated nearby the river. We placed 2 plates under the lights with some ethanol 70% and every 10 minutes we replaced the plates for empty ones. Adults were counted separating males and females to obtain the sex ratio. When the quantity of adults was too much to be counted in 10 minutes we placed them into 250 ml plastic recipients with 70% ethanol and they were counted in the laboratory. The capture effort was always the same, using the same light intensity and the same light direction to the river.

Results

Larval density and life history

The high density obtained in late May decreased steeply in mid June and when emergences began, from late July to the end of the sampling period, when the larval population was reduced to few individuals per m^2 (Fig. 4). Survivorship curves in 2005 had the same pattern as in 1987 but shifted (see Ibañez et al. 1991).

Growth curves in 2005 showed a steady increase from May to early July and a marked increase to late July, when larvae reached a growth peak and attained the maximum average size. Individual size ranged from 4 to 8 mm in May growing very fast until 18-20 mm in the peak of July (Fig. 5). That is, growth was exponential until late July (r^2= 0.94) and during August larvae had slightly lower body size and dry weight. As shown in Fig. 6, wing pad length and head width also rose steeply in July, in close relationship with other growth parameters, indicating that the growth peak corresponded to the larvae about to emerge. When in late July larvae sex could be identified, we observed that females were bigger than males, being around 5 mm longer (Fig. 7). At this time the sampled population was composed of 57% female and 43% males while on 4th August the larvae population was formed mainly of males (70 %) and on 12th August females predominated (66.6 %). The density of larvae during late July and August was very low and the collection of just a few specimens was even difficult.

Between-year differences in BL and DW were strongly marked for the same period. As can be seen in Fig. 5, mean BL obtained in 2005 for early July was 10.9 ± 3.5 mm, while in 1987 BL did not reach 9.2 ± 3.7 mm until July 30th. The same pattern was observed for individual dry weight, in 1987 larvae reached an average of 3.02 mg the 7th August, while in 2005 the same values were obtained one month before in 4th July, so the maximum size and weight was attained earlier in 2005 than in 1987.

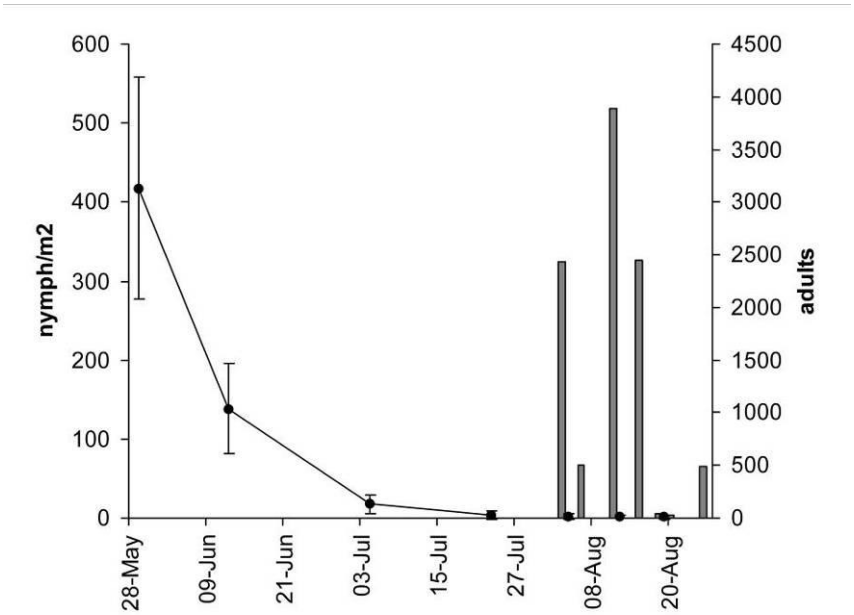


Figure 4. Mean larval densities and standard deviations, and number of adults (bars) captured during emergences in 2005.

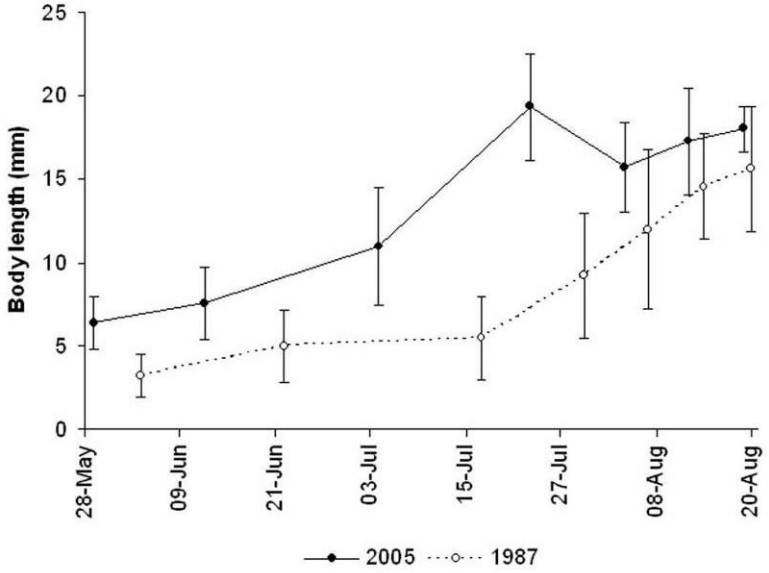


Figure 5. Mean body length (mm) and standard deviation of the larvae during 2005 and 1987.

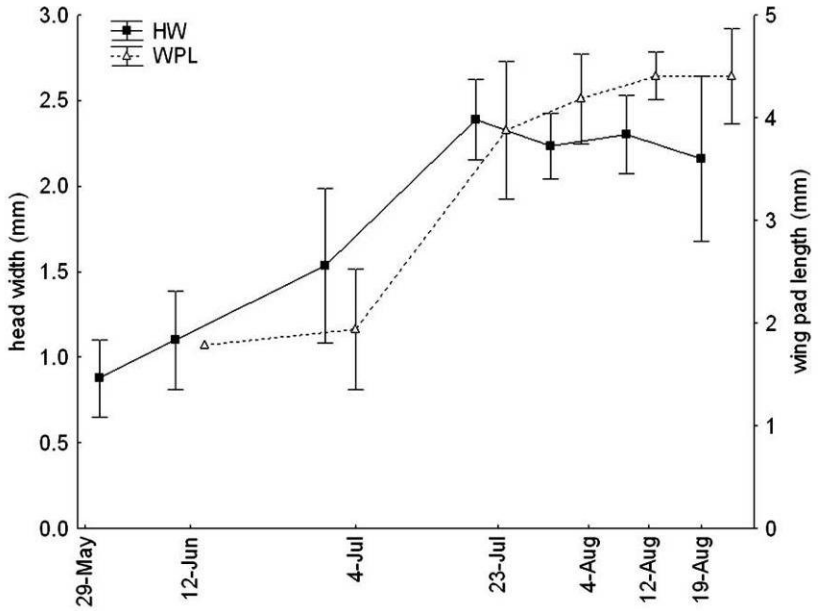


Figure 6. Mean and standard deviation of head width (HW) and wing pad length (WPL) during 2005.

BL was positively correlated with head width (Spearman r= 0.93, p=0.00) following a lineal relationship (BL= 6.9 HW +0.3) and a potential relationship (DW= 0.0004 BL $^{3.6769}$) with DW (Spearman r= 0.98, p=0.00).

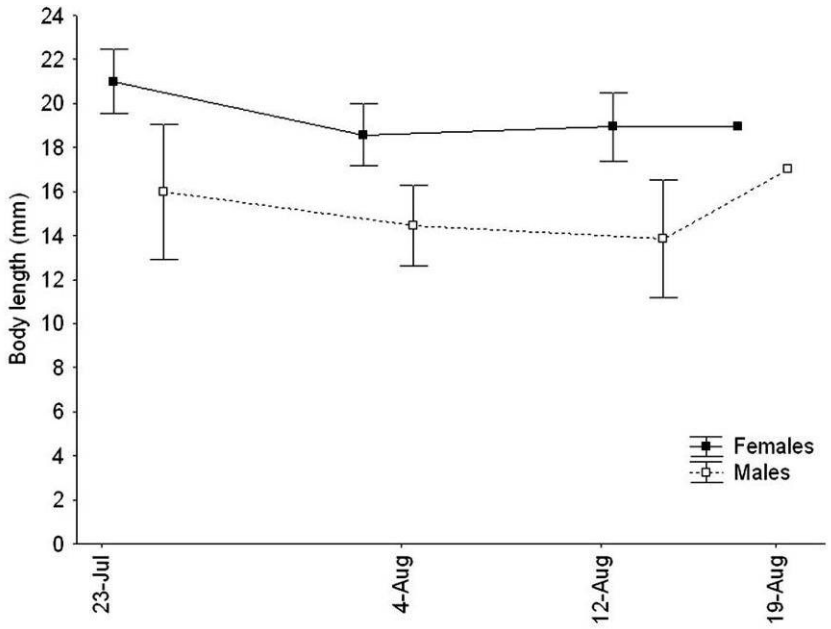


Figure 7. Mean body length (mm) and standard deviations of females and males during last stages of development in 2005.

Emergence began in late July and ended in late August. Estimated dates of peak emergence were earlier in 2005. As shown in Fig. 4, the most important number of adults were captured before August 15th (of a total 9.842 individuals captured, 9264 were from August 3rd to 15th), while in 1987 the peak emergence period lasted until early September (Escosa et al. 1989). We observed that emergences were very low when the weather was adverse (windy or stormy nights). We obtained a sex ratio (M: F) of 1:4 in 2005, female biased, while in 1987 the adult population was male biased, with a sex ratio of 2:1. Daily sex ratio varied depending on the sampling day but in 2005 was always female dominated (Fig. 8). The emergence pattern was sex dependent; while males appeared during the first 10 min, females emerged 20 min later (Fig. 9), except in mid August when the number of captured adults was just a few individuals. During emergences the presence of insectivorous fishes, birds and bats feeding on *E. virgo* was constant.

Production estimates

As shown in table 1 similar values were obtained for the annual production with the IS (950.42 mg· m^{-2}· y^{-1}) and RS (1079.78 mg· m^{-2}· y^{-1}) method. The annual turnover ratio (P/B) ranged from 10.11 to 11.49 y^{-1}. The average population biomass during the entire sampling period was of 93.98 mg·m^{-2}, being high in the initial stages due to abundant larvae densities and falling drastically when emergence began.

To be comparable to 2005, we applied the correction factor for the effects of preservation to the 1987 data of larval weight. In 1987 production was also similar using the two different methods but IS and RS values were less than half the production obtained in 2005 (Table 1).

Temperature

For the entire larval growth period (April-August) in 2005 we obtained a total of 1157 DD using the daily mean from fifteen minute intervals. From mid April (hatching) to the first emergence sampled in early August we estimated a total of 827.4 DD. To compare the water temperatures of 2005 with the ones of 1987 we could not use the fifteen minute interval data and the comparison was made with the available records of mean monthly temperatures for both years multiplied by the number of days. With this method for 2005 we obtained similar DD (1152 DD) accumulated from April to August than if fifteen minute data were used. For the larval period of 1987 we obtained a total of 1043 DD, a slightly lower value than in 2005. Month by month along the sampling period DD accumulation was always higher in 2005 (Fig. 10). A significant correlation (r Spearman= 0.91) between air and water temperatures in the period of 1996-2005 was found, therefore, air temperature may be used to estimate water values. From May to July 2005 mean daily air temperatures were almost 2 ºC higher than in 1987 (Fig. 11). Thus, higher air and water temperatures during the larval period were obtained for 2005 compared with previous data from 1987.

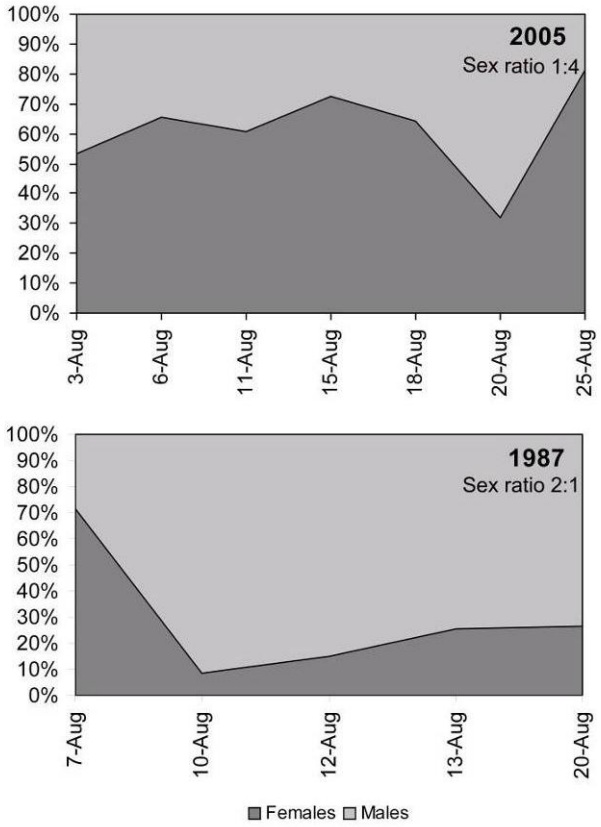


Figure 8. Percentage of adult males and females emerged in 2005 and 1987.

Table 1. Average and total biomass (B), production (P) and P: B ratio using 2 methods for 2005 and 1987. All values were corrected for preservation procedures. Methods described are IS: Increment summation; and RS: Removal summation.

	2005	1987	Unit
Average B	93,98	78,74	$mg \cdot m^{-2}$
Total B	657,89	551,18	$mg \cdot m^{-2}$
Annual P (IS)	950,42	440,76	$mg \cdot m^{-2} \cdot y^{-1}$
Annual P (RS)	1079,78	444,1	$mg \cdot m^{-2} \cdot y^{-1}$
Annual P/B ratio (IS)	10,11	5,60	y^{-1}
Annual P/B ratio (RS)	11,49	5,64	y^{-1}

Methods described are IS, increment summation; RS, removal summation.

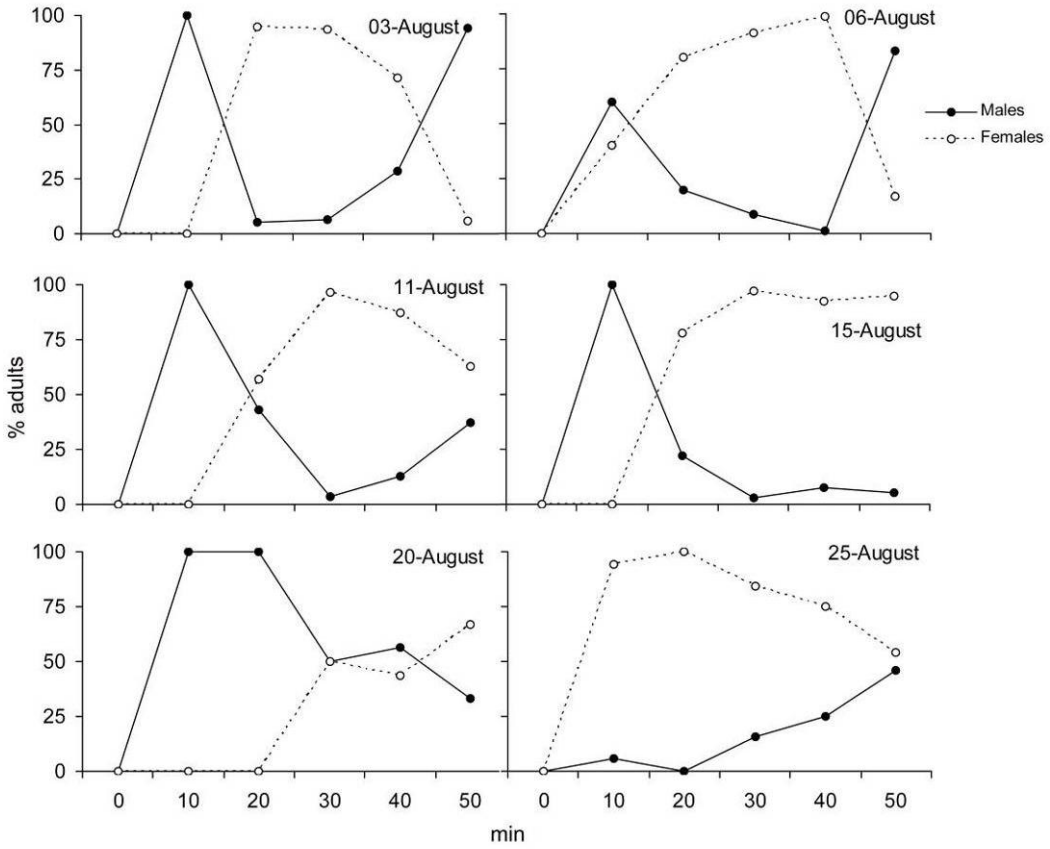


Figure 9. Percentage of adult males and females emerged in 2005 at 10 minute intervals.

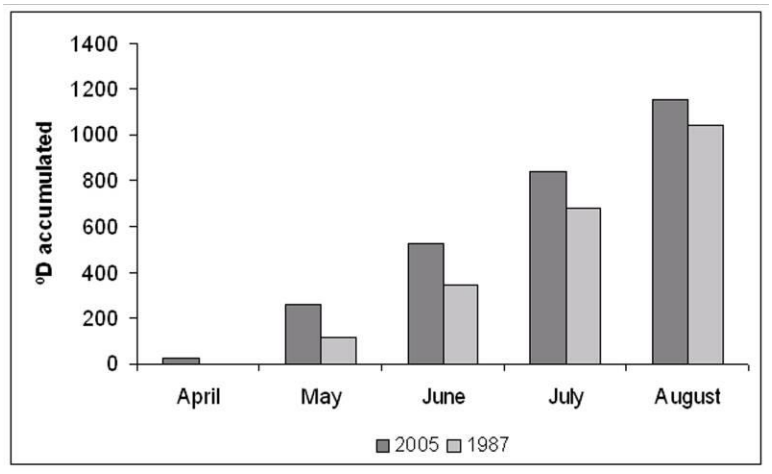


Figure 10. Degree days accumulated for 2005 and 1987 calculated from CHE (Confederación Hidrográfica del Ebro, Ebro Water authority).

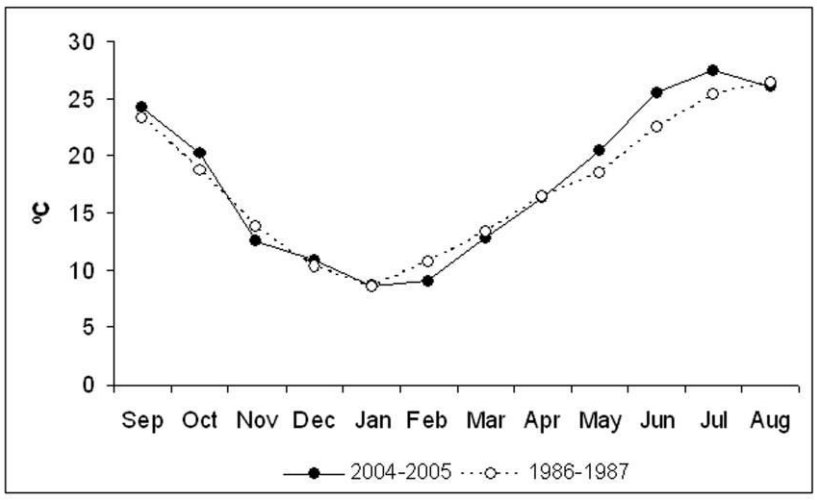


Figure 11. Average monthly air temperatures for 2005 (black dots and lines) and 1987 (white dots and discontinuous lines).

Discussion

In species with a marked synchronization such as in mayflies like *E. virgo*, different methods for production calculation should give similar values (Morin et al. 1997). According to this, similar production estimates were obtained by using IS and RS methods (Table 1).

Secondary production in the Ebro River in 2005 was higher than in 1987, but not reaching the high values known from other *Ephoron* species as *Ephoron leukon* (Williamson, 1802) and *Ephoron album* (Say, 1824) in North America (Table 2). In 1987 lower production values were obtained partly due to the higher male proportion with lower individual body mass. Population density during the first sampling collection in 1987 was underestimated because higher river discharge did not allow using the Surber sampler, only the kick net. Therefore low biomass and less production were obtained during the initial sampling period in 1987. Another factor that could explain the lower production in 1987 was a longer storage period. In 1987 larvae were stored for 30 months in ethanol 70%, while in 2005 storage was for 6 months, so the weight loss in larvae corresponding to 1987 could have been higher and this may be one of the reasons why lower production values were obtained.

Once emergences had begun, growth curves showed that larval body length and individual mass decreased, corresponding to larvae that were still ending the last instar

development in August. This pattern of growth decline after first emergences has also been observed in other Ephemeroptera such as *Euthyplocia hecuba* (Hagen, 1861) (Sweeney et al. 1995) and *Ephoron shigae* (Takahashi, 1924) (Watanabe & Ohkita 2000) and seems to be temperature independent. Female larvae of *E. virgo* are bigger than male ones with marked differences in body length and mass (Kureck & Fontes 1996), due to egg accumulation in the female abdomen, so a higher proportion of females in the sampled population could result in higher larval mean sizes. That is, the lower larval body sizes found in 4th August could be due to a male biased sex ratio.

Emergence and oviposition behaviour was the same described for *E.virgo* populations in the Rhine River (Kureck & Fontes 1996) and similar to other *Ephoron* species such as *E. album* (Giberson & Galloway 1985), *E. leukon* (Snyder et al. 1991) or *E. shigae* (Watanabe et al. 1999, Watanabe & Ohkita 2000).

According to Benke (1984) the most important factor limiting production in rivers where food is not a limiting factor is habitat characteristics, so production would be optimal when the functional habitat per unit area is high. Habitat available for *E. virgo* in the lower Ebro river has been reduced during the past 5 years due to the invasion of the macrophyte pondweed *Potamogeton pectinatus* L. As the macrophyte community is being established, they accumulate soft sediments in the habitat they occupy, changing river sediment and hydraulic conditions (Cotton et al. 2006; Wharthon et al. 2006; Sand- Jensen 1998) so it is possible that areas *E. virgo* used to colonise nowadays are not a suitable habitat for the species. The decrease of dissolved phosphorous in all the drainage basin in the past 10 years is likely the cause of the observed phytoplankton reduction (Ibañez et al. in press), affecting the food availability of *E. virgo*. The presence of the zebra mussel *Dreissena polymorpha* (Pallas, 1771) in the Riba-Roja dam, upstream the sampling zone, could have also enhanced a decrease of total suspended materials by its filtering action. At the same time, populations of the Asian clam *Corbicula fluminea* (Müller, 1774) and the black fly *Simulium erytrocephalum* (De Geer, 1776) are well consolidated in the river, so they could compete with *E. virgo* for the same food resources. However, despite these possible competitors for food, higher production estimates were found in the studied area, giving the impression that food is not a limiting factor in this river. However, *E. virgo* production will probably decrease in the future due to habitat constraints (disappearance of gravel areas that will

be covered by silt and organic matter debris accumulated below *Potamogeton pectinatus* stands).

Table 2. Secondary production comparison of *Ephoron virgo* with other *Ephoron* species. Methods described are IS: Increment summation; RS: Removal summation; IG: Instantaneous growth. SF: Size frequency.

Author	Species	Study site	Annual P ($mg/m^2/y$)	Annual mean B (mg/m^2)	Annual P/B (y^{-1})	Method
Cid. et al. (present study)	*Ephoron virgo*	Lower Ebro river	950.42	93,98	10,11	IS
			1079.78		11,49	RS
Ibañez et al. (1991)	*Ephoron virgo*	Lower Ebro river	440.76	78,74	5,60	IS
			444.1		5,64	RS
Snyder et al. (1991)	*Ephoron leukon*	South river	398–2857	99–911	15.3–13.5	IS
Gibberson and Galloway (1985)	*Ephoron album*	Valley river	1430		22.8	RS
			1320		21.2	IG
			1320		21.2	AC
			1480		21.3	SF
Phillips et al. (1994)	*Ephoron album*	Ilinois river	5919	340	17.26	RS
			6698		19.52	IS
			6097		17.77	IG

Methods described are IS, increment summation; RS, removal summation.

Factors regulating growth and development in aquatic insects are mainly determined by water temperature, food quality and availability and competition (Sweeney & Vannote 1978; Vannote & Sweeney 1980; Ward & Stanford 1982; Rader & Ward 1989; Snyder et al. 1991; Atkinson 1994; Hogg & Williams 1996). These factors have a direct influence on larval size before emergence, on emergence timing, population densities and consequently on secondary production. Taking into account that density in the 1987 first sampling was underestimated due to methodological problems (small larvae not detected and the use of the kick net), we can conclude that larval densities followed the same pattern in 2005 but advanced some weeks. That is, the marked population decrease coinciding with the initial emergences occurred in mid July in 2005 and in August in 1987, so the life cycle in 2005 was advanced three weeks. Since maturity and emergence depend on size and weight reached in a certain moment (Snyder et al. 1991; Rowe & Ludwig 1991), the early development of the larvae in 2005 agrees with the early emergences found. Several studies have shown changes in timing of life cycle

events related with warming. Hogg & Williams (1996) observed that emergences of the stonefly *Nemoura trispinosa* (Claassen, 1923) and the caddisfly *Lepidostoma vernale* (Banks, 1897) were advanced two weeks with a water temperature increment of 2ºC in spring and 3.5ºC in summer, and also Langford (1975) noticed that in warm years with higher water temperatures first emergences of caddisflies and mayflies were advanced. The development of *E. virgo* studied in Morocco (Qninba 1986) started earlier than in the Ebro river in 1987 (Ibañez et al. 1991) due to warmer water temperatures and a different thermal regime. Moreover, an advanced emergence of terrestrial insects has also been reported in the lower Ebro, due to an increase of air temperature since the mid 1970ies, especially in spring (Gordo & Sanz 2005). Since the larval growing period of *E. virgo* mostly takes place in spring, a similar response is expected for this aquatic insect. Therefore, after 18 years higher water temperatures were likely the main cause of the shifted life cycle. Since both studies (1987 and 2005) were performed when the hydropower dams and the nuclear power station had already been built, and knowing that the study site is more than 50 km downstream, where no influence on temperature exists, we assume that water temperature increase is a result of warmer air temperatures. If the global trend of increasing temperatures is maintained we will find that life history parameters (timing of hatching and emergences) of this species may still change in the future. For this reason, experimental works to determine the effects of temperature increase on the species are required. Also data on the habitat preferences of *E. virgo* in the Ebro River and a more extended study of production along different parts of the river will be needed to determine how habitat influences the population dynamics of this species.

Acknowledgements

This study was funded by the Government of Catalonia (Agència Catalana de l'Aigua and Departament d'Innovació, Universitats i Empresa), the European Social Fund, and the Government of Spain (Ministerio de Educación y Ciencia, research project CGL2006-01487, Plan Nacional I+D+I). We thank the Ebro Water Authority (Confederación Hidrográfica del Ebro) and the Ebro Observatory for the water and air temperature data used in this paper.

References

Atkinson D. 1994. Temperature and organism size – a biological law for ectotherms? Advances in Ecological Research 25: 1-58.

Benke AC, Van Arsdall TC, Gillespie DM, Parrish FK. 1984. Invertebrate productivity in a subtropical blackwater river: the importance of habitat and life history. Ecological monographs, 54: 25-63.

Benke A C. 1993. Edgardo Baldi Memorial Lecture, Concepts and patterns of invertebrate production in running waters. Verhandlungen der Internationalen Vereinigung für Limnologie 25: 15-18.

Buffagni A, Comin E. 2000. Secondary production of benthic communities at the habitat scale as a tool to assess ecological integrity in mountain streams. Hydrobiologia, 422: 183-195.

Cotton J A, Wharton G, Bass JAB, Heppell CM, Wotton RS. 2006. The effects of seasonal changes to in-stream vegetation cover on patterns of flow and accumulation of sediment. Geomorphology 77: 320-334.

Escosa R., Ibàñez C, Muñoz I, Prat N. 1989. Informe final sobre *Ephoron virgo* ("La palometa") al Baix Ebre. Aspectes ecològics i solucions pràctiques per a evitar les aglomeracions a la ciutat de Tortosa. Technical report.

Giberson DJ, Galloway TD. 1985. Life history and production of *Ephoron album* (Say) (Ephemeroptera: Polymitarcidae) in the Valley river, Manitoba. Canadian Journal of Zoology 63: 1668-1674.

González J M, Basaguren A, Pozo J. 2003a. Life history and production of *Epeorus torrentium* Eaton (Ephemeroptera: Heptageniidae) in a North Iberian stream. Aquatic insects 25: 247-258.

González J M, Basaguren A, Pozo J. 2003b. Life history and coexistence of two leptophlebiid mayflies in three sites along a Northern Spain stream. Archiv für Hydrobiologie, 158: 303-316.

Gordo O, Sanz JJ. 2005. Phenology and climate change: a long- term study in a Mediterranean locality. Oecologia 146:484-495.

Hogg I D, Williams DD. 1996. Response of stream invertebrates to a global-warming thermal regime: an ecosystem- level manipulation. Ecology 77: 395- 407.

Ibàñez C, Escosa R, Muñoz I, & Prat N. 1991. Life cycle and production of *Ephoron virgo* (Ephemeroptera: Polymitarcidae) in the lower river Ebro (NE Spain). In: Aba-Tercedor J, Sanchez-Ortega A, editors. Overview and Strategies of Ephemeroptera and Plecoptera. The Sandhill Crane Press, USA. p 483-492.

Ibáñez C, Prat N, Duran C, Pardos M, Munné A, Andreu R, Caiola N, Cid N, Hampel H, Sánchez R, Trobajo R. In press. Changes in dissolved nutrients in the lower Ebro river: causes and consequences. Limnetica.

Iversen T M, Thorup, J. 1987. Population dynamics and production of *Sialis lutaria* L. (Megaloptera) in the Danish river Susa. Freshwater Biology 17: 461- 469.

Kureck A, Fontes RJ. 1996. The life cycle and emergence of *Ephoron virgo*, a large potamal mayfly that has returned to the river Rhine. Archiv für Hydrobiologie Supplement 113: 319-323.

Langford TE. 1975. The emergence of insect from a British river, warmed by power station cooling-water. Part II. The emergence patterns of some species of Ephemeroptera, Trichoptera and Megaloptera in relation to water temperature and river flow, ustream and downstream of the cooling-water outfalls. Hydrobiologia 47: 91-133.

Lutgthart GJ, Wallace, JB. 1992. Effects of disturbance on benthic functional structure and production in mountain streams. Journal of the North American Benthological Society 11:138-164.

Mérigoux S, Dolédec S. 2004. Hydraulic requirements of stream communities: a case study on invertebrates. Freshwater Biology 49: 600-613.

Morin A, Mousseau TA, Roff DA. 1987. Accuracy and presicion of secondary production estimates. Limnology and Oceanography 32: 1342-1352.

Muñoz I, Prat N. 1994. Macroinvetebrates in the lower Ebro river (NE Spain). Hydrobiologia 286: 64-78.

Phillips EC, Kilambi RV, Carlton CE. 1994. Life history and secondary production of *Ephoron album* (Say) (Ephemeroptera: Polymitarcyidae) in the Illinois river, Arkansas. Journal of the Arkansas Entomological Society 67: 242-247.

Prats J, Val R, Armengol J, Dolz J. In press. Temporal scales of variability of the thermal regime in the lower Ebro River (Spain) and its alterations due to anthropogenic factors. River Research and Applications.

Qninba A. 1986. Structure, dynamique et microdistribution de quelques peuplements d'invertebrés benthiques de l'oued Bouregreg (Plateau central marocain) [dissertation]. Rabat (Morocco): University Mohamed V. Rabat.

Rader R B, Ward JV. 1989. Influence of impoundments on mayfly diets, life histories and production. Journal of the North American Benthological Society 8: 64-73.

Rigler F H, Downing JA. 1984. The calculation of secondary productivity. In: Downing JA, Rigler FH, editors. A manual on methods for assessment of secondary productivity in fresh waters. Oxford, United Kingdom: Blackwell. p 19-54

Rowe L, Ludwig D. 1991. Size and timing of methamorphosis in complex life cycles: time constraints and variation. Ecology 72: 413-427.

Sand- Jensen K. 1998. Influence of submerged macrophytes on sediment composition and and near- bed flow in lowland streams. Freshwater biology 39: 663-679.

Scrimgeour GJ. 1991. Life history and production of *Deleatidium* (Ephemeroptera: Leptoplebiidae) in an unstable New Zealand river. New Zealand Journal of Marine and Freshwater research 25: 93-99.

Snyder CD, Willis LD, Hendricks AC. 1991. Spatial and temporal variation in the growth and production of *Ephoron leukon* (Ephemeroptera: Polymitarcidae). Journal of the North American Benthological Society 10: 57-67.

Southwood TRE. 1978. Ecological methods, with particular reference to the study of insect populations. 2nd ed. London: Chapman and Hall. 524 pp.

Stief P, Altmann D, de Beer D, Bieg R, Kureck A. 2004. Microbial activities in the burrow environment of the potamal mayfly *Ephoron virgo*. Freshwater Biology 49: 1152-1163.

Sweeney BW, Vannote RL. 1978. Size variation and the distribution of hemimetabolous aquatic insects: two thermal equilibrium hypotheses. Science 200: 444-446.

Sweeney BW, Jackson JK, Funk DH. 1995. Semivoltinism, Seasonal Emergence, and Adult Size Variation in a Tropical Stream Mayfly (*Euthyplocia hecuba*). Journal of the North American Benthological Society 14: 131-146.

Van der Geest HG, Soppe WJ, Greve GD, Kroon A, Kraak MHS. 2002. Combined effects of lowered oxygen and toxicants (copper and diazinon) on the mayfly *Ephoron virgo*. Environmental Toxicology and Chemistry 2: 431-436.

Vannote RL, & Sweeney BW. 1980. Geographic analysis of thermal equilibria: a conceptual model for evaluating the effect of natural and modified thermal regimes on aquatic insect communities. The American Naturalist 115: 667-695.

Ward JV, Stanford JA. 1982. Thermal responses in the evolutionary ecology of aquatic insects. Annual Review of Entomology 27: 97-117.

Watanabe NC, Mori I, Yoshitaka I. 1999. Effect of water temperature on the mass emergence of the mayfly *Ephoron shigae* in a japanese river (Ephemeroptera: Polymitarcyidae). Freshwater biology 41: 537-541.

Watanabe NC, Ohkita A. 2000. Life cycle and synchronization of larval development of the mayfly *Ephoron shigae* in Japan (Ephemeroptera: Polymitarcidae). Aquatic insects 22 (2): 108-121.

Waters TF, Crawford GW. 1973. Annual production of a stream mayfly population: a comparison of methods. Limnology and Oceanography 18: 286-296.

Wharton G, Cotton JA, Wotton RS, Bass JAB, Heppell CM, Trimmer M, Sanders IA, Warren LL. 2006. Macrophytes and suspension-feeding invertebrates modify flows and fine sediments in the Frome and Piddle catchments, Dorset (UK). Journal of Hydrology 330: 171-184.

Chapter 4

Patterns of metal bioaccumulation in two filter-feeding macroinvertebrates: Exposure distribution, inter-species differences and variability across developmental stages.

Cid, N., Ibánez, C., Palanques, A. & Prat, N. 2010.

***Science of the Total Environment* 408 (3): 2795–2806.**

Patterns of metal bioaccumulation in two filter-feeding macroinvertebrates: exposure distribution, inter-species differences and variability across developmental stages

Núria Cid[1,3], Carles Ibáñez[1], Albert Palanques[2] and Narcís Prat[3]

[1] *Aquatic Ecosystems, IRTA, Carretera Poble Nou, Km 5.5, P.O.Box 200, E-43540 Sant Carles de la Ràpita, Catalonia, Spain.*
[2]*Institut de Ciències del Mar, CSIC, Passeig Joan de Borbó s/n, 08039 Barcelona, Catalonia, Spain.*
[3] *Departament d'Ecologia, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, E-08028 Barcelona, Catalonia, Spain.*

Corresponding author. Tel.: 0034- 93 40 11 88; Fax: 0034- 93 411 14 38

E-mail address: ncid@ub.edu

Abstract

This study focused on the metal bioaccumulation of two aquatic insects (*Ephoron virgo* and *Hydropsyche spp*.) in order to evaluate the spatial distribution of metals, the interspecific differences between both filter –feeders and the bioaccumulation dynamics during *E. virgo* development stages. Hg, Cd, Ni, Cr, As, Pb, Cu, Ti, Zn and Mn were quantified in insects and in suspended particulate matter (SPM) sampled downstream and upstream of a chemical plant, where more than 300,000 t of polluted sediments are deposited. Hg concentrations were one order of magnitude higher downstream of the sediment dump, which showed that the Hg pollution originated in the chemical plant. Cd, Ni, Cr, Pb, Ti, Zn and Mn in invertebrates revealed that metal pollution was present upstream in other parts of the river. Interspecific differences were observed for all metals but Mn; significantly higher concentrations were observed in *E. virgo* over *Hydropsyche exocellata*, except for Cd, which showed 10-fold higher values. Hg and Cd increased until *E. virgo* nymphs reached 11 mm and decreased afterwards in late instars when nymphs were about to emerge. Cr, Pb, Ti and Mn decreased along early instars followed by a steady state in late instars. Similar values were obtained for Cu, As and Zn along all instars. Sexual differences between males and females of *E. virgo* were observed for Cd, Cu and Mn. Hg and Cd persistence was strong across developmental stages since high concentrations were found in eggs and emerging adults. Because the behavior of different metals varied for the two species and during the developmental stages of *E. virgo*, care should be taken in the interpretation of insect metal concentrations when analyzing the food chain transfer of metals in river ecosystems.

Keywords: Heavy metals; Bioaccumulation; *Ephoron virgo*; *Hydropsyche*; life cycle; Ebro River

1. Introduction

The analysis of metal bioaccumulation on biota is necessary to evaluate the relevance of metal pollution in aquatic ecosystems (Zhou et al., 2008) and insect larvae have been widely used for this purpose either from population-community or at species level (Chapman et al., 2003). Furthermore, the body metal concentration in insect larvae is useful to detect metal bioavailability (Goodyear and McNeill, 1999) since aquatic invertebrates are relatively sedentary and complete all or most of their life cycle in the aquatic environment. Studies conducted in streams and rivers using aquatic invertebrates are mainly related to mine pollution (Cain et al., 2000; Solà et al., 2004) or urban/ industrial activities (Beauvais et al., 1995). Most studies involving severe metal pollution use tolerant insects, such as larvae of several species of the caddisfly *Hydropsyche.* These species are used since their populations are abundant and widespread and can be present in highly metal polluted areas (Clements et al., 2000). In contrast, rivers or reaches of a river with moderate metal pollution offer the opportunity to study the variability in bioaccumulation between different taxa (Cain et al., 2004), e.g., sensitivity of different species, comparing the same feeding guilds, or contributing to a better understanding of the metal- and species-specific responses of aquatic insects in field conditions. Metal concentrations in aquatic insects also change with size and life cycle stages and different bioaccumulation patterns have been observed depending on the metal in question (Smock, 1983). However, the present knowledge of these patterns is incomplete since these studies focused on only a few metals at a time. The element-specific behavior of metals across the developmental life cycle of insects remains unclear. Moreover, studies have demonstrated that, for two species (*Hexagenia rigida* and *Stenacron interpunctatum*), variability exists in bioaccumulation of certain metals (cadmium, mercury, lead, chromium and zinc) within an insect's body, occurring internally in gut tissues or externally in the exoskeleton (Smock, 1983; Hare et al., 1991; Inza et al., 2001). However, this finding has not been widely tested among other insect species or across a broader group of metals.

The lower Ebro River receives a permanent influx of heavy metals mainly due to point source pollution originating from a chemical plant located in the town of Flix (100 km from the river mouth). Since the completion of the Flix Reservoir in 1949, and until the 1980s, more than 300,000 t of polluted industrial solid wastes from alkali-chlorine electrolysis and phosphate fertilizer production have been deposited in the reservoir

(Palanques et al., 1996). Previous studies indicate the presence of a large variety of pollutants (heavy metals and organochlorine compounds) in reservoir sediments (Grimalt et al. 2003) and detect heavy metal bioaccumulation downstream in the aquatic food web from the Ebro Delta (Schuhmacher et al., 1993; Mañosa et al., 2001; Sánchez-Chardi et al., 2007) and from the Flix Reservoir (Carrasco et al., 2008). However, there is a lack of information on metal distribution and bioavailability in the first 60 Km downstream of the sediment dump, corresponding to the freshwater part of the river.

Since the metals deposited in the Flix Reservoir are very insoluble in water, they are present in fine sediments that can be released downstream as suspended particulate matter (SPM). Our main objective was to assess if the contaminated sediments in the Flix Reservoir are transported downstream and transferred to the aquatic food web. Two aquatic insects, *Ephoron virgo* (Ephemeroptera: Polymitarcyidae) and *Hydropsyche spp.* (Trichoptera: Hydropsychidae) were selected as indicator species, because both are representative of our study area(the Ebro River), have the same feeding guild as filter-feeders (Tachet et al., 2000) and inhabit areas of running water. Thus, their selection avoids variability in bioaccumulation due to habitat constraints or to different feeding habits. While hydropsychids are considered tolerant of heavy metals (Cain et al., 2004; Solà et al., 2004), *E. virgo* is considered metal-sensitive (De Haas et al., 2002).

In this context, the spatial patterns of metal contamination in the two organisms should reflect the higher metal concentrations downstream of the factory, at least for the metals detected at high concentrations by Grimalt et al. (2003) (i.e., Hg, Cd, Ni Cr and Zn). Additionally, our goal was to evaluate interspecific differences of metal bioaccumulation in two invertebrate species of the same functional feeding group. Finally, the metals bioaccumulated during the nymph growth stage of *E. virgo* and along all the stages of its life cycle were measured, including adult moults, adult males, adult females and eggs.

2. Materials and Methods

2.1 Study Site description

The Ebro River is located in the NE Iberian Peninsula and has a drainage basin of 85,550 km^2 with a length of 928 km. The lower part of the river (100 km from the river

mouth) has a mean annual flow of 426 $m^3 \cdot s^{-1}$ and is regulated by two main hydropower dams (Mequinença and Riba-Roja) constructed in the late 1960s. The Ebro basin is impacted by industrial, urban and agricultural activities from its headwaters to its mouth and the presence of metals and organic compounds have been reported in many sites along the river (Lacorte et al. 2006; Terrado et al., 2006).

As our study focuses on the possible transport of heavy metals downstream of Flix, the macroinvertebrates were sampled at three sites along the river downstream of the chemical plant (Station A: Ascó; Station M: Móra; Station T: Tortosa). Station A is located 8 km downstream of the waste dumping site in Flix and Stations M and T are 21 km and 68 km downstream, respectively. These stations were located in the freshwater part of the river, without the influence of the salt wedge present downstream (see Guillén et al., 1992; Ibáñez et al., 1997 for a detailed description of the area and its hydrological characteristics).

True reference sites without any heavy metal pollution upstream of Flix are difficult to find in the Ebro basin. Although measures of dissolved heavy metals in water are low for all sites according to the reports of the Ebro Water Authority (Annex 1, supporting data), no or scarce data exists on suspended sediments. We selected three control stations for heavy metal concentrations upstream of Flix. The first control station is located in the middle part of the Matarranya River, a tributary of the Ebro River (Station MAT). While this station is probably the closest to true reference conditions, only *Hydropsyche* larvae may be found here.

The second control station (Station MZ) is located in the main Ebro River at the town of Monzalbarba. We selected Monzalbarba due to its location 12 km upstream from the industrialized area of Zaragoza, thereby avoiding pollution inputs from this large city at the control station. We recognize that low to moderate industrial and urban pollution inputs from the upper part of the river may occur at the station. However, this was the only site upstream of the Flix Reservoir that contained a sufficiently large population of *E. virgo* for larval sample comparisons with Station T.

Finally, a third station was selected in the confluence of the Segre and Cinca Rivers in the Aiguabarreig area (Station AB). These two tributaries of the Ebro River are impacted by large-scale agricultural areas. The Cinca River is also impacted by the industrialized area of Monzón (located 80 km upstream). The selection of MZ and AB

upstream stations would allow us to distinguish which metals come from other sources in other parts of the basin and which ones come form the Flix reservoir.

Particulate matter was sampled at three stations (Station F: Flix; Station M: Móra; Station R: Riba-roja). Station F is located in the Flix Reservoir, close to the chemical plant; Station M coincided with one of our previous affected sites for invertebrates downstream of Flix; and Station R is a control site located in the reservoir upstream from the chemical plant. The two invertebrates sampled were not present at Stations R and F due to habitat constraints (they are not present in deep- low water velocity areas of the reservoirs). Water quality data and metal concentration in water for several of the stations in Fig. 1 are shown in Annex 1 (supporting data).

2.2 Sampling of suspended particulate matter and macroinvertebrates

Water samples from the river were taken during spring and summer 2006 and 2007. They were filtered through pre-weighted 0.22 μm pore cellulose filters, where SPM was retained. The volume of water taken depended on the sample and on the saturation of the filter used.

E. virgo nymphs and larvae of the genera *Hydropsyche* were collected during June 2006 and 2007 using a kick net (250 μm mesh net). Since more than one species of *Hydropsyche* could be found upstream, all the species were grouped at the genus level when mentioned in the text, except for sites where only one species can be found. The species found in the Ebro River is *Hydropsyche exocellata* (Muñoz and Prat, 1994), corresponding to the affected sites downstream of the sediment dump and the control Station MZ. At Station AB, *H. exocellata* was also the only hydropsychid species found. However, at control Station MAT, three species of Hydropsychids were cohabitating: *H. exocellata*, the dominant species (50% of all Hydropsychids found), *H. incognita* and *H. bulbifera*. The species were classified from samples of the macroinvertebrate community at each sampled site. Although most of the hydropsychids examined were *H. exocellata*, we grouped them as *Hydropsyche spp*. at Station MAT since more than one species was found. We assumed that grouping them as *Hydropsyche spp*. at Station MAT would not generate any significant bias in the results. We based this assumption on the similar Cu concentrations obtained in three

Hydropsyche species by Cain et al.(1992) (*Hydropsyche* sp., *H. occidentalis* and *H. cockerelli*) and on the results of Buchwalter et al. (2008), who obtained similar efflux rates of metals for *H. californica* compared with those for *H. betteni* (Evans et al., 2002).

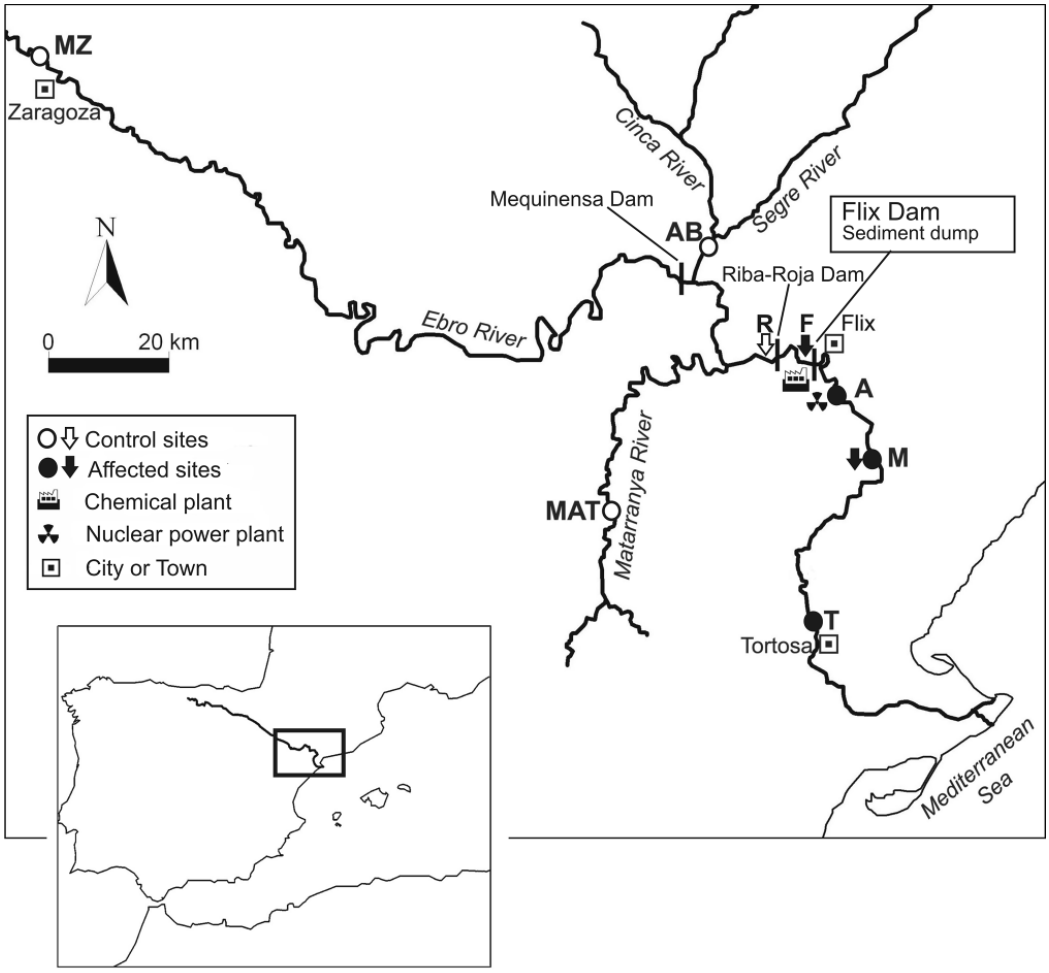


Fig. 1. Sampling sites and study area at the Ebro River basin (NE Iberian Peninsula). Circles show sampling sites for invertebrates and arrows for suspended particulate matter. (MZ: Monzalbarba; AB: Aiguabarreig; MAT: Matarranya; R: Riba-Roja; F: Flix; A: Ascó; M: Móra; T: Tortosa).

The riverbed was disturbed and the subsequent sample was washed into the net and deposited in a tray to collect individuals of both macroinvertebrates. Once the organisms were sorted, they were cleaned with double distilled water, placed in acid-washed Eppendorf vials and stored with ice, following the methodology developed by Solà et al. (2004). Due to the low abundance of *E. virgo* at some areas of the river, this species could be sampled only in one control station (MZ) and one affected station (T).

In order to compare the interspecific differences in bioaccumulation, only the samples from the same affected station (T) in the same sampling period (2007) were chosen to avoid possible spatial and interannual differences. Thus, the species compared were *E. virgo* and *H. exocellata*.

In order to study metal bioaccumulation patterns during nymph growth and life cycle stages, *E. virgo* samples were taken from Station T, where the characteristics of life cycle and secondary production of this species are well known (Ibáñez et al., 1991; Cid et al., 2008). Nymphs at different stages of development were sampled in late May, June, July and August for 2005, and in June for 2006 and 2007 with the same methods explained above. When adult emergences began (early August), adults were also sampled by light trapping. Adult females and males, male moults and eggs extracted from females were collected separately. Triplicate samples were composed by approximately 7-8 individuals each one in order to obtain a minimum weight to analyze (values ranged from 5 mg to 150 mg). At the laboratory, all samples were frozen at -20ºC until analyses were carried out.

2.3 Metal analyses

For SPM samples, a two-step bulk sample digestion method was used according to Querol et al. (1996). It comprises the digestion of volatile elements in a closed system with concentrated HNO_3 (MERK supra-pure) at 90^{o}C for two hours and the digestion of non-volatile elements with supra-pure HF and heating at 90^{o}C for three hours and the addition of supra-pure $HClO_4$ and HNO_3. Macroinvertebrate samples were freeze dried, weighted to the nearest 0.001 mg and oven digested in closed Teflon vials with HNO_3 and H_2O_2 (high purity reagents) according to the procedure described by Solà et al. (2004). Vials were placed in an oven at 90ºC for 10 hours to obtain the sample solution and the digestion solution was diluted with double-distilled (Milli Q) water.

Hg, Cd, Ni, Cr, As, Pb, Cu, Ti, Zn and Mn were selected for analysis because these metals were detected by Grimalt et al. (2003) in sediments from the Flix Reservoir and the lower Ebro River. For SPM, Ti, and Zn were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES), and Cd, Ni, Cr, As, Pb, Cu, and Mn by inductively coupled plasma mass spectrometry (ICP-MS). The concentration of Hg

in SPM was measured using a Leco AMA 254 analyzer. The macroinvertebrate metal analysis was carried out using ICP-MS with Rh as the internal standard. The quality control of all processes included blanks and the analysis of standard reference material. For SPM, digestions of MESS-3 and PACS-2 reference materials from the National Research Council of Canada were used. Within these samples, the percent recovery of this reference material was <10% for all the analyzed elements. For the invertebrates, the reference material was GBW08572-Prawn for *E. virgo* in 2005 and ERM-CE278-mussel tissue for all samples in 2006 and 2007. To compare the measurement with the certified value, a guide from the European Reference Materials was used, which takes into account uncertainties and compares the expanded uncertainty to obtain the difference (Linsinger, 2005). According to this guide, ERM-CE278- mussel tissue did not show significant differences between the certified and measured values for Hg, Cd, As, Pb, Cu, Zn and Mn; for Cr, there was a significant overestimate, with a measured value double the certified value. For GBW08572-Prawn, Cr and Pb were significantly different than the certified value; Pb was undervalued and Cr was overvalued, as occurred in ERM-CE278- mussel tissue. This lead to the question whether Cr is an adequate metal to be analyzed by the previous methods or not. No reference material was available for values of Ni and Ti, so we could not test the quality control of the analysis for these metals.

2.4 Statistical analysis

In samples where metal concentration was below the detection limit, values used for statistical analyses were half the value of the detection limit (Karouna-Renier and Sparling, 2001).

In order to test differences among sites for both macroinvertebrate species, to assess possible differences in interspecific metal concentrations and to determine the different life cycle bioaccumulation patterns of *E. virgo* an analysis of variance (ANOVA) was performed. When necessary, a Games-Howell post-hoc test (hereafter, GH tests) was conducted. GH tests are post-hoc multiple comparison tests that are among the most powerful and robust for unequal variances (Day & Quinn, 1989). Variables were transformed for parametric analyses because homoscedasticity and linearity were clearly improved. Statistical analyses were performed with SPSS 16.0.

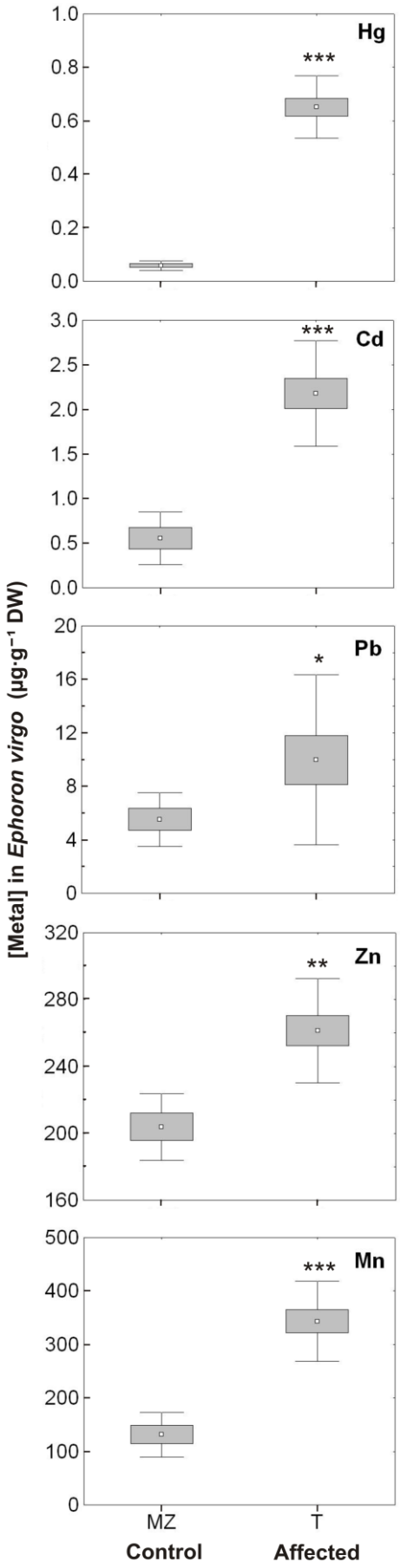
3. Results

3.1. Distribution of heavy metal pollution

Hg concentration in SPM at Station F was more than two orders of magnitude higher than at control Station R, while Cd concentrations were 10-fold higher at Station F and two-fold higher at Station M (Table 1). Ni, Cr, As, Pb, Cu, Ti, Zn and Mn concentrations did not show any relevant metal enrichment at Stations F and M, relative to the upstream Station R.

Table 1. Metal concentration (μg· g^{-1} DW) in suspended particulate matter (SPM) from the Ebro River. Km indicates the distance from pollution source of the affected stations. For station codes see figure 1.

Station	***Description***	***Date***	***Hg***	***Cd***	***Ni***	***Cr***	***As***	***Pb***	***Cu***	***Ti***	***Zn***	***Mn***
R	Control	04/04/06	0.17	0.26	33.1	74.48	15.9	47.0	56.78	2730	176.2	1039.0
		04/04/07	0.07	0.27	44.5	105.8	21.8	30.5	34.6	3119	151.9	573.0
F	0 km	04/04/06	7.24	4.66	37	88.17	16.05	45.9	60.12	2580	227.2	1430.0
		31/05/06	26.93	4.48	44.2	185.4	19.3	25.9	69.1	1450.0	234.0	1141.0
		18/07/06	31.14	1.51	30.7	125.9	16.4	47.2	49.8	1345.0	125.0	3535.0
M	21 km	04/05/06	1.66	0.45	33.5	93.2	18.4	30.8	46.3	2345.0	173.6	674.0
		27/06/06	1.53	0.58	39.8	90.6	19.3	31.5	41.2	2178.0	168.8	655.5
		03/04/07	1.86	0.38	41.2	97.4	20.7	27.8	43.3	2894.0	156.6	753.0
		26/06/07	1.88	0.69	40.7	98.6	20.1	29.6	45.5	2235.0	148.9	648.5



Spatial distribution of metal concentrations in SPM generally reflected those in the invertebrates for Hg and Cd. Concentrations of Hg and Cd in *E. virgo* nymphs downstream of the chemical plant were significantly higher than upstream at control Station MZ (Fig. 2). Hg concentration of this mayfly downstream of the sediment dump at Station T site were more than 10-fold higher than at Station MZ, followed by Cd, with a mean concentration four-fold higher. In *Hydropsyche,* concentrations of Hg and Cd showed a general tendency to be higher downstream of the Flix chemical plant at Stations A, M, and T (Fig. 3). Hg concentrations downstream of Flix were significantly higher than all the upstream sites ($P<0.05$). The highest concentration was found at Station A, where mean values were up to 10-fold higher than upstream, similar to the results found for *E. virgo*. Cd was higher than all control sites only at Station M ($P<0.01$); levels found at control Station MZ were comparable to those at Stations A and T.

Fig. 2. Metal concentration of *Ephoron virgo* nymphs at affected and control sites (symbol: mean; box: SE; whiskers: SD; *ANOVA, $P<0.05$; ** ANOVA, $P<0.01$; *** ANOVA, $P<0.001$). For station codes see Fig. 1.

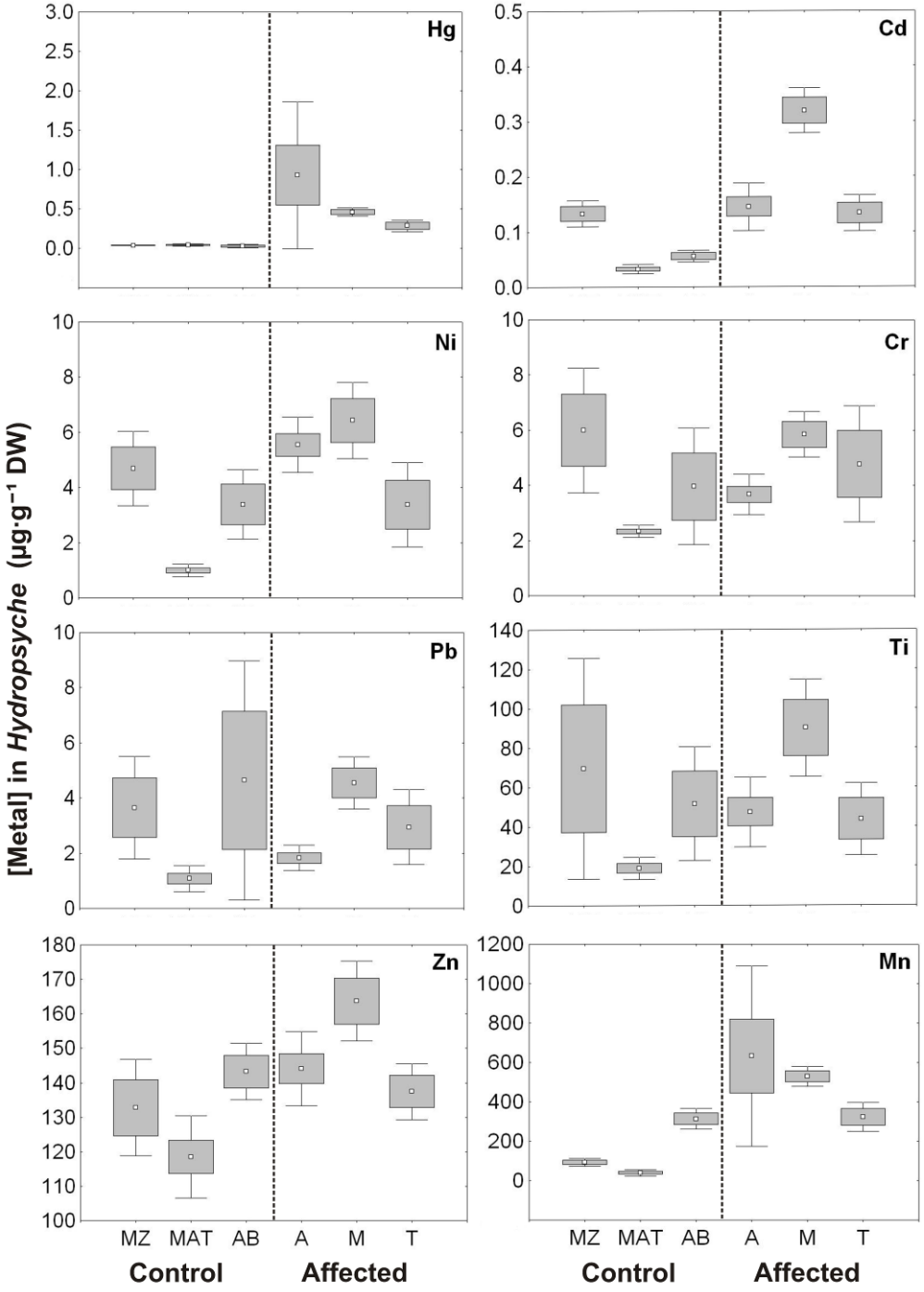


Fig. 3. Metal concentration of *Hydropsyche* at affected and control sites (symbol: mean; box: SE; whiskers: SD). For station codes see Fig. 1.

The other metals presented different results depending upon whether *E. virgo* or *Hydropsyche* were analyzed at the sampling stations. Pb, Zn and Mn in *E. virgo* samples downstream at Station T were significantly higher than upstream at control Station MZ (Fig. 2), and the same pattern was observed for Hg and Cd. However, similar values

were found for Ni, Cr, As, Cu and Ti concentrations in this organism (Annex 2, supporting data).

In contrast, Pb and Zn in *Hydropsyche* at control Station MZ presented similar results as the affected sites below Flix (Annex 3, supporting data), except for Mn, which showed higher values than those at control Stations MZ and MAT ($P<0.05$). Although similar concentrations of Ni, Cr, Pb, Ti and Zn were found upstream at control Stations MZ and AB compared to downstream sites, higher concentrations relative to control Station MAT were reported ($P<0.05$). As noted above, control Station MAT was considered closest to reference conditions. Therefore, as we suspected, the spatial distribution of metal pollution reflected by *Hydropsyche* metal concentrations indicated that the only sampled site not impacted by metals was control Station MAT. Conversely, no significant differences in As and Cu were observed between any of the stations.

3.2. Interspecific differences in bioaccumulation

Metal concentrations at the same sampling station (T) between the two organisms of the same feeding guild were significantly higher in the mayfly *E. virgo* than in the caddisfly *H. exocellata*, except for Mn (Fig. 4). Most of the metals bioaccumulated by *E. virgo* were double the concentrations of *H. exocellata*, but the most marked difference was observed for Cd, with *E. virgo* values one order of magnitude higher (Annex 4, supporting data). Therefore, the differences of metal bioaccumulation between *E. virgo* and *H. exocellata* appear to be stronger for Cd than for other metals.

3.3. Metal bioaccumulation patterns along Ephoron virgo nymph growth

Although statistical differences were not found for metal concentrations in nymphs at different instars, several trends were observed for the studied metals. One pattern was related to the group of Hg and Cd, in which mean metal concentration showed a steady increase in 2005 until nymphs reached a mean body length of 11 mm and decreased afterwards when their size was 16 mm (Fig. 5). This coincides with the period in which late instars were about to emerge. Another pattern was related to Pb, which showed a decrease in concentration during the early instars followed by a steady state until the nymphs finished growing. Metals following a similar pattern to Pb were Cr, Ti and Mn (Annex 5, supporting data). Finally, similar values were obtained for Cu, As and Zn

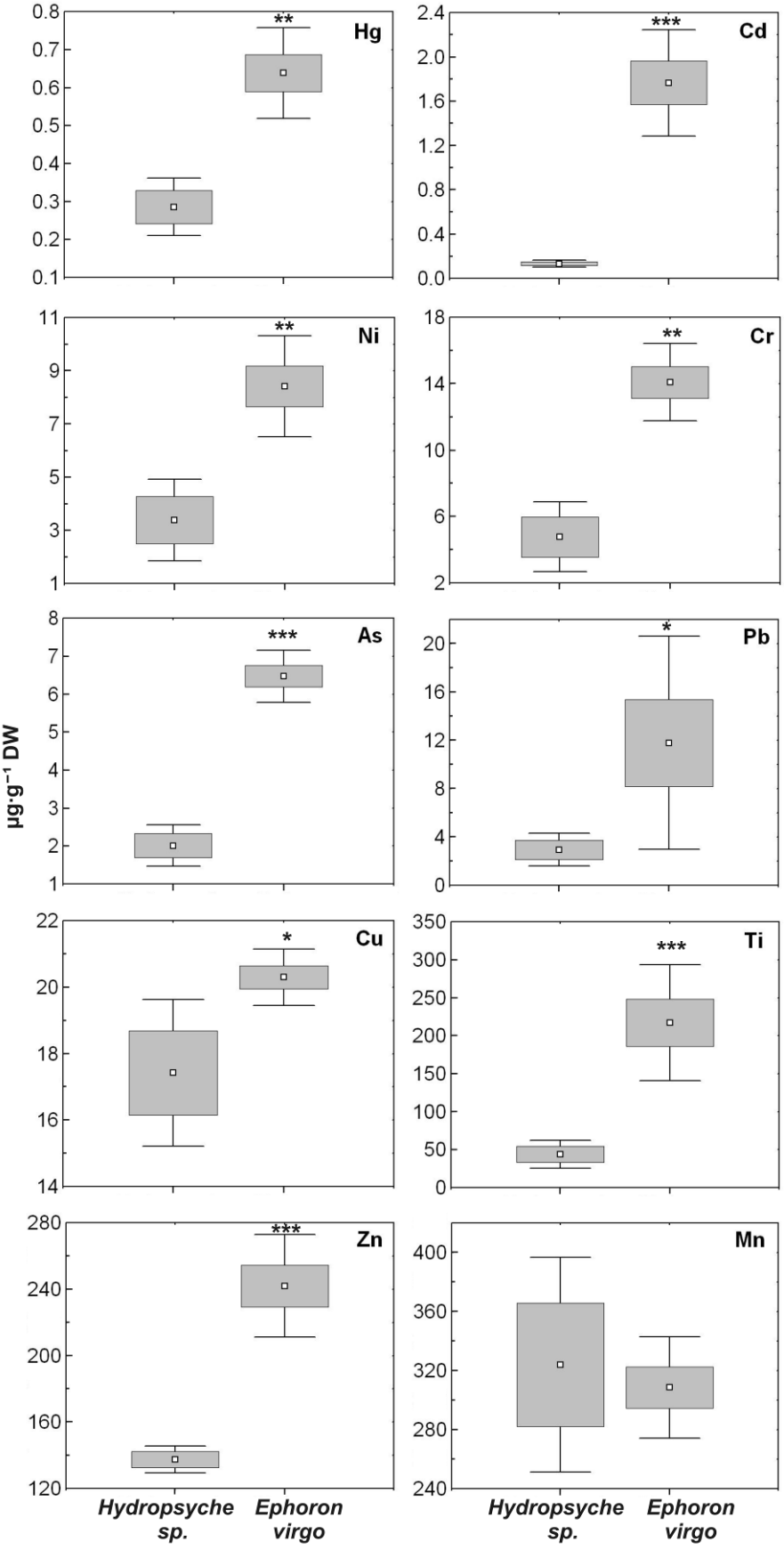


Fig. 4. Metal concentration (µg· g^{-1} DW) of *Hydropsyche exocellata*. and *Ephoron virgo*. Data were collected at the same affected site (T) and the same day in 2007. (*ANOVA, $P<0.05$; ** ANOVA, $P<0.01$; *** ANOVA, $P<0.001$).

along all nymph instars,. For Ni, no patterns were able to be deduced since Ni was not analyzed in 2005.

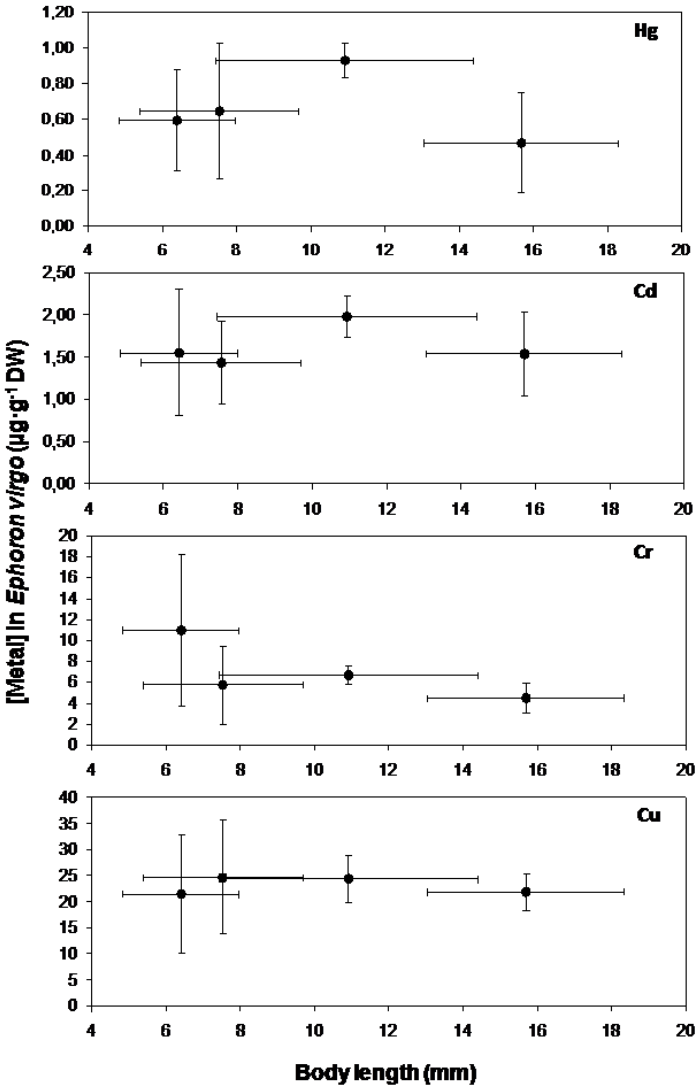


Fig. 5. Metal concentration of *Ephoron virgo* along nymph growth at the T affected site. Values are means from 2005 (symbol: mean; bars: SD).

At the two sampling events conducted in 2006 and 2007, the data only showed a slight increase in Hg and Cd in nymphs in 2007 (0.55±0.06 to 0.73±0.01 $\mu g \cdot g^{-1}$ for Hg and 1.35±0.11 to 2.18±0.11 $\mu g \cdot g^{-1}$ for Cd), corroborating the pattern observed in 2005, while in 2006 similar values were observed. In 2006 and 2007, Cr, Pb, Ti and Mn again showed a concentration decrease in growing nymphs, and similar values were also obtained for Ni, As, Cu and Zn.

3.4 Metal bioaccumulation patterns along Ephoron virgo life cycle stages

When comparing metal concentrations throughout the different stages of the life cycle, nymphs always had the highest values ($P<0.05$), except for Pb and Cu values, which were highest in the male moult (Fig. 6). Nymphs had significantly higher concentrations than eggs for all metals but Ni ($P<0.001$). When emergence occurs, females and males emerge as subimago (prior stage to become an adult), however males moult to an imago (adult) in the shore vegetation for mating while females mate and ovoposit as subimago. Therefore, we compared the metal concentrations from the male moult and the adult male in order to test the metal lost through moulting. Cd concentration in adult males was significantly higher than in moults ($P<0.01$), while for Ni, Cr, Pb and Mn, moults had higher concentrations than adult males ($P<0.01$). No significant differences were observed for Hg, As, Cu, Ti and Zn, although Hg was near the significance limit ($P= 0.08$) to have a higher concentration in adult males than in the moult.

Sexual differences in metal concentration were tested, and statistical differences were obtained only for Cu and Mn concentrations, with higher levels of Cu in adult males than in females ($P<0.05$), and higher concentrations of Mn ($P<0.01$) in females than in males. Although no significant differences in Cd concentration were observed between adult males and females, females had half the value of males.

Metal concentrations in eggs and in the whole female (including eggs in the abdomen) were compared and several differences in metal bioaccumulation were obtained. Hg ($P<0.05$), Cd ($P<0.001$), Cu ($P<0.001$) and Zn ($P<0.01$) in eggs were significantly lower than in females, while for Cr, Ni, As, Pb, Ti and Mn similar values were obtained. However, Hg concentrations in eggs were not as low as other metals since eggs had half the value of adults.

The metal concentrations of nymphs and adults were compared in order to evaluate whether the risk of metal transfer to higher trophic levels during emergence was similar to the nymph period. Hg was the only metal showing adult male and female concentrations similar to nymphs. Adult females had significantly lower concentrations of Cd than nymphs ($P<0.001$), but not adult males. For the other metals analyzed (Ni, Cr, As, Pb, Ti, Zn and Mn), nymphs had always greater concentrations than adults ($P<0.05$).

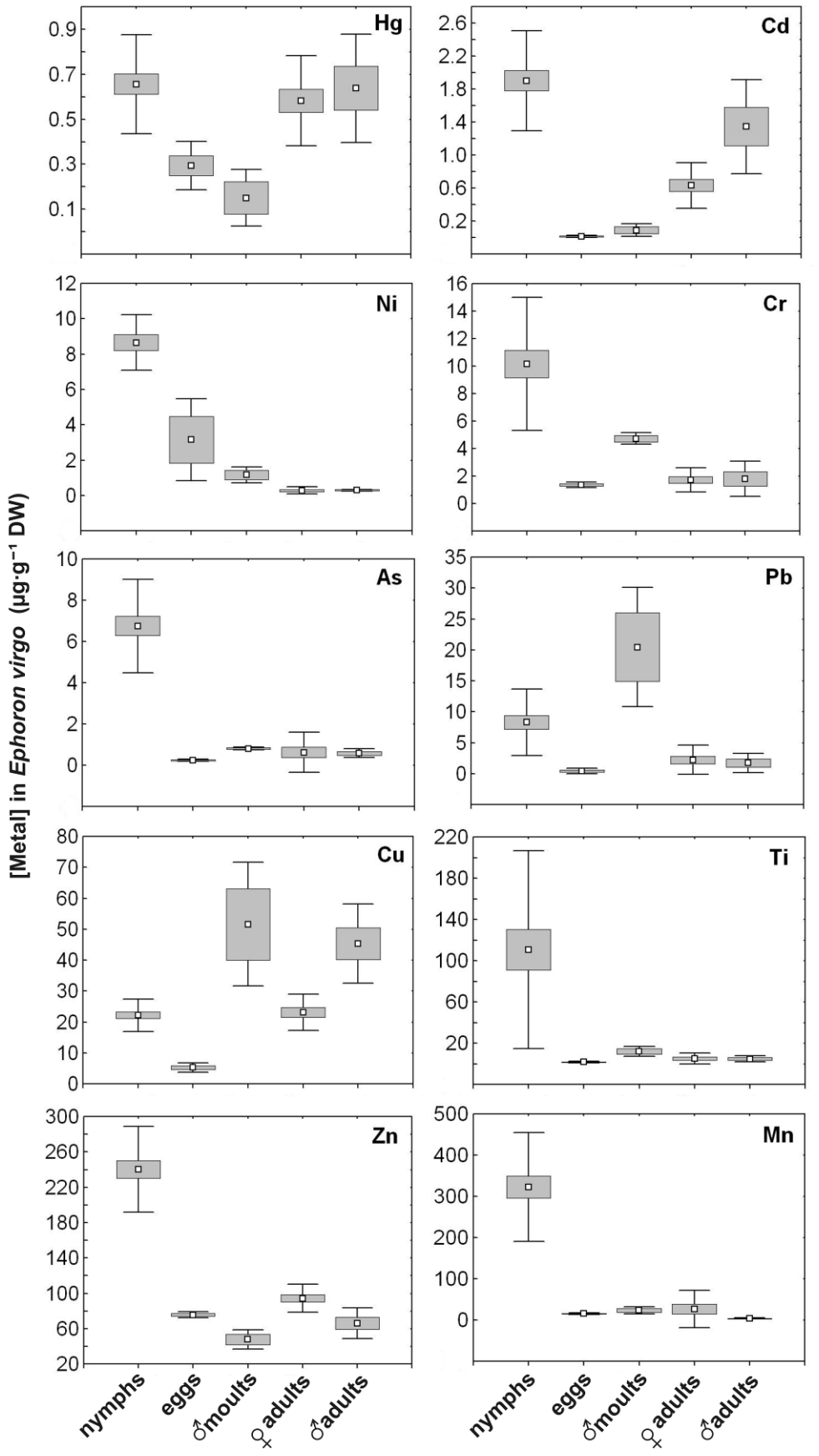


Fig. 6. Metal concentration of *Ephoron virgo* at different stages of development at the T affected site. Values are means from 2005, 2006 and 2007(symbol: mean; box: SE; whiskers: SD).

4. Discussion

4.1. Distribution of heavy metal pollution

The metal concentrations found in SPM and in macroinvertebrates reflect the transport of Hg and Cd in the lower Ebro to approximately 60 km downstream of the Flix dam. The SPM in the Flix Reservoir had very high Hg and Cd concentrations and their levels were still elevated in SPM samples at Station M downstream of the Flix dam, exceeding the concentrations at the upstream Station R (Table 1) and those of natural unpolluted sediments (see Palanques et al. (1990) for levels of unpolluted fine sediment transported by the Ebro River). As a result, high Hg and Cd exposures were detected in macroinvertebrate samples downstream of Flix, indicating an impact from the reservoir.

Ni, Cr and Zn concentrations in SPM at Station M were also high compared to natural Ebro River sediments and showed similar concentrations relative to Station R upstream, indicating that SPM at Station R was contaminated with these metals. Together with the fact that exposures to Cd, Ni, Cr and Zn by the invertebrates did not exceed those at some upstream control sites (e.g., Station MZ), these results confirm that these metals are present in many parts of the basin, stemming from other anthropogenic activities (Terrado et al., 2006, Ramos et al., 1999). Thus, site-specific concentrations in organisms indicate that the origin of Hg pollution came mainly from the transport of the solid wastes from the chemical industry in Flix, which uses Hg as a cathode cell in electrolysis processes, whereas Cd, Cr and Zn come both from the chemical industry in Flix (attributed to the phosphorite used as raw material) and from upstream.

Although experimental studies are needed to describe the processes involving metal bioaccumulation in aquatic insects, the high concentrations of Hg and Cd in the organisms and in the SPM suggested that metal exposure in the lower Ebro River ecosystem can occur via the ingested SPM transported downstream of Flix because SPM is the main food supply for these organisms. Since the benthic habitat of *E. virgo* and *H. exocellata* is not likely to be contaminated because the high water velocities do not allow SPM to be deposited, the hypothesis that metals are incorporated from the transported SPM can be supported. Moreover, metal pollution has been reported before in the lower Ebro sediments and food web. In previous studies of sediment pollution (Palanques et al., 1996; Grimalt et al., 2003), concentrations of Hg, Cd, Cr, Ni and Zn in the Flix Reservoir exceeded those from natural fluvial sediments and some metals

(mainly Hg and Cd) were also detected downstream at some depositional areas of the river. Hg also appeared in the Ebro Delta marine mollusks and algae (Schuhmacher et al. 1993), in birds feeding on fish (Mañosa et al., 2001), and in insectivorous mammals (Sánchez-Chardi et al., 2007). Recently, within the context of the project where the present study was carried out, high Hg concentrations were detected in zebra mussels (*Dreissena polymorpha*) from the Flix Reservoir (Carrasco et al., 2008), and levels of Hg and Cd in European catfish (*Silurus glanis*) and carp (*Cyprinus carpio*) measured higher downstream of Flix than upstream (unpublished data).

As, Pb, Cu, Ti and Mn concentrations in SPM were similar to those from natural unpolluted sediments at all sampling sites, showing no evidence of metal pollution. These levels match sediment samples from the Flix Reservoir (Palanques et al., 1996; Grimalt et al., 2003), in which As and Cu concentrations were slightly higher than in natural sediments and Pb, Mn and Ti were not considered to be at pollution levels. As and Cu in *E. virgo* and *Hydropsyche* reflected these concentrations in SPM and sediments since no significant differences were observed in any of the sampling sites. However, the high Pb, Ti and Mn concentrations in macroinvertebrates downstream of Flix did not reflect those in the SPM, suggesting that the exposure could be from dissolved metals, and that these metals occur locally at different points upstream and downstream of Flix. The Mn variability in the macroinvertebrates could be attributed to other causes as it is also a diagenetically mobile element (Van Cappellen and Wang, 2006) and may be affected by the reduction-oxidation processes in the bottom of Riba-roja Reservoir.

Comparing our results with other metal pollution impacted areas, Hg whole body concentrations in *E. virgo* were seven-fold higher than the mean concentration in Mississipi River mayflies *Hexagenia sp*. (Ephemeroptera: Ephemeridae) affected by urban/ industrial pollution (Beauvais et al., 1995). In contrast, mayflies and caddisflies from the Idrija River (Slovenia) had very high Hg concentrations, up to two orders of magnitude higher than those from the Ebro River, due the severe pollution caused by a mercury mine (Žižek et al., 2007).

Cd mean concentrations in *E. virgo* in our study were much higher than in the Mississipi River *Hexagenia sp*. (Beauvais et al., 1995). However, compared with metal-mining impacted rivers, our Cd results were much lower than those found in the Clark

Fork River, USA, for the mayflies *Epeorus albertae* and *Serratella tibialis* (Cain et al., 2004). Also, Cd concentrations in *Hydropsyche* from the affected sites in the Ebro were much lower than those reported for Hydropsychids in mine-impacted rivers (Cain et al. 1992, 2000, 2004; Maret, 2003; Solà et al., 2004). Hydropsychids from the Ebro River had lower Zn and Pb levels in comparison to those in Maret et al. (2003). However, the values we report are close to the ranges obtained for Hydropsychids of the metal mining-polluted Clark Fork River (Cain et al., 1992) and the Sacramento River, USA (Cain et al., 2000), indicating a high level of Zn and Pb pollution in the Ebro.

4.2. Interspecific differences in bioaccumulation

It is widely known that different species living and feeding in the same habitat can bioaccumulate different amounts of metal (Luoma and Rainbow, 2005), due to their different physiology and detoxification mechanisms (Hare, 1992). Several experimental studies have revealed that different species of aquatic macroinvertebrates have different metal elimination rates. High Cd elimination rates have been reported in metal tolerant species, such as *Hydropsyche californica* (Buchwalter et al. 2008) and *Hydropsyche betenni* (Evans et al. 2002), so similar results would be expected for *H. exocellata*. In contrast, as low elimination rates were observed in sensitive mayflies, such as *Maccaffertium ithaca* and *Rhithrogena morrisoni* (Buchwalter et al., 2008), *Ephemerella excrucians* (Buchwalter et al. 2007) and *Hexagenia rigida* (Hare et al 1991), *E. virgo* could have a similar response to metals. As explained by Buchwalter et al. (2008), these elimination rates are phylogenetically linked. However, since in our field study more than one metal was present and the environmental conditions were not controlled, the resulting data is more complex to interpret. Since diet is likely the predominant exposure route for these organisms, other determinants that might influence the bioaccumulation variability within two species of the same feeding guild include feeding rate, concentration and assimilation efficiency (Luoma and Fisher, 1997). For instance, very high metal concentrations have been reported in marine invertebrates with fast ingestion rates, high assimilation efficiencies and slow elimination rates (Luoma and Rainbow, 2005). Thus, the higher metal bioaccumulation observed in *E. virgo* compared to *H. exocellata* might be a result of a particularly low elimination rates,accordingly to its phylogenetical position, together with a differential feeding rate. The particle size of the ingested material by each species, the specific body size effect and absorption in the gut (McLachlan, 1996) may be other factors to consider

for further studies, together with the use of more than one tolerant and sensitive species at several sampling sites.

4.3 Element- specific patterns of bioaccumulation in Ephoron virgo

This study showed three different patterns of metal bioaccumulation as *E. virgo* nymphs were growing, depending on the metal in question. Firstly, Hg and Cd concentrations slightly increased from early to late instars, except in nymphs about to emerge, when the concentrations decreased. The slight increase of Hg and Cd in *E. virgo* developing nymphs could be related to the metal site of storage, the path of exposure and the physiological response to these metals. Studies on metal bioaccumulation from diet in the mayfly *Hexagenia rigida* showed that Hg, Cd and Zn were mainly located in the gut tissues (Hare et al., 1991; Saouter et al., 1993; Inza et al., 2001), so a similar pattern could be expected in *E. virgo*. As suggested previously, if Hg and Cd are ingested from SPM, the decline in the last instar could be due to a shift in diet during this period, i.e., the nymphs can ingest larger particles with lower metal concentrations compared to particles ingested by early instars, which could be metal-enriched, as described by Smock (1983). One hypothesis was that no relevant Hg and Cd loss occurred in relation to instar change (moulting) because metals were not present in the exoskeleton. For these reason, the concentration ratio "subimago male moult/imago male" was used as an indicator of metal lost by moulting. Ratios near zero were expected when no metal loss via exoskeleton occurred. The lowest male moult/male ratio was obtained for Cd, followed by Hg (Table 2). The minor concentration of these metals exhibited in the subimago moult suggests that the majority of Hg and Cd in *E. virgo* was present internally (gut components).

Secondly, Cr, Pb, Ti and Mn concentrations in nymphs tended to decrease with organism size, as was also observed in the mayflies *Stenodesma modestum* and *Stenacron interpunctatum* for Cr (Smock, 1983), suggesting that those metals could be present in the exoskeleton and consequently lost in each instar moult. In our study we must consider that Cr concentration was overvalued when compared to the reference material, however, even though the measured concentrations are overvalued, the patterns of bioaccumulation as nymphs grow can also be compared. Cr, Pb, Ti and Mn were associated with a subimago male moult/imago male ratio higher than 2 (Table 2), meaning that the concentration of these metals was much higher in the moult than in the

adult male. This ratio provides evidence for metal loss via exoskeleton, at least in the step from subimago to imago. Some studies on mayfly growth and metal bioaccumulation suggest that instar change could be involved in the decontamination process (Jop and Wojtan, 1982; Smock, 1983; Jop, 1991), although this would depend on the metal analyzed and the exposure route (diet or solution). The adsorption of metals to the exoskeleton has also been previously reported in mayflies, whether the metal was dissolved in water, as Cr (Smock, 1983), or in sediments, as Pb (Hare et al., 1991). There is also evidence that pathways are present to move bioaccumulated metals to the exoskeleton in order for the insects to use the metals in their cuticular tools (Schofield et al., 2003). Therefore, variability exists in the way the metals could be transferred to the exoskeleton in *E. virgo*, depending on the exposure route and the internal processes during development.

Table 2. "Egg/subimago female" and "subimago male moult/ imago male" ratios from mean values of metal concentration in *Ephoron virgo.*

Ratio	**Hg**	**Cd**	**Ni**	**Cr**	**As**	**Pb**	**Cu**	**Ti**	**Zn**	**Mn**
male moult/ male	0.24	0.07	4.02	2.61	1.40	11.87	1.14	2.50	0.72	5.86
eggs/females	0.50	0.02	11.26	0.80	0.38	0.19	0.23	0.33	0.80	0.58

Finally, Ni, As, Cu and Zn showed similar values for different nymph instars and no clear patterns could be described. Cu and Zn had a subimago male moult/imago male ratio close to 1, suggesting that these metals may partially accumulate in the exoskeleton but also be present in other parts of the body. However, Ni seemed to be located mainly in the exoskeleton because higher values in the moult than in the male were observed (subimago male moult/imago male ratio= 4).

Since this study was conducted under field conditions, we must consider the variation attributed to the SPM released from the Flix Reservoir, since the SPM available is dependent on the fraction that escapes from the reservoir and the dilution and dispersion downstream. In other words, the amount of metal available at each moment while nymphs are growing can affect our results. For this reason, further studies under controlled laboratory conditions are required to describe in more detail the bioaccumulation patterns during *E. virgo* growth.

4.4 Sexual variability of Ephoron virgo emergent adults in bioaccumulation

Sexual differences in Hg and Cd bioaccumulation in emergent mayflies have been described by Dukerschein et al. (1992). They pointed out that the low concentrations found in females might be due to the large egg masses that contribute to the dilution of the metal concentration, which implies that concentration in eggs should be very low in comparison to females. This means that females hardly transfer the metals to eggs and that eggs can be a protected compartment that is not in equilibrium with metal concentrations in the female body. In our study, the egg/ subimago female ratio was used as an indicator of egg contribution to adult female dilution of metals. In general, the lower the ratio, the higher should be the dilution by eggs. In the present study, sexual variability in bioaccumulation was only considered for Cu, Mn and Cd. The low Cd concentrations in females could be explained by the very low egg/ subimago female ratio (Table 2), indicating a possible dilution by eggs. The low Cu concentration in females can be only partly attributed to egg dilution since Cu had a ratio 10 times higher than Cd. Nevertheless, sexual differences in bioaccumulation can also be caused by other factors, as a differential moulting process. Considering the biology of *E. virgo*, in which females emerge as subimagos and males as imagos, males could have lost metals by their last moulting when the metal is mainly located in the exoskeleton and have final concentrations lower than females (e.g., in Mn concentrations).

4.5 Other ecological implications related with E. virgo life cycle and metal bioaccumulation

E. virgo has a synchronized life cycle (Kureck, 1996; Cid et al. 2008) and mass emergences occur during summer in the lower Ebro River. When this occurs, the amount of biomass that is present in the sediment emerges and becomes easily available to higher trophic levels, including predators living in the river or in the riparian and terrestrial ecosystems. Since Hg and Cd concentrations in emergent adults were similar to those in nymphs, exposures to these metals through insectivores feeding on adults (as fish, amphibians, bats, etc.) could be important during the short *E. virgo* emergence period, as seen in other studies of emergent insects (Dukerschein et al., 1992; Currie et al., 1997). Other metals that may be a risk in the lower Ebro ecosystem because of the high concentrations found in nymphs (Ni, Cr, Pb, Ti and Zn) had adult metal

concentrations much lower than nymphs, so exposure to these metals during emergence would be lower.

Hg and Ni present a higher risk than other metals due to their persistence in *E. virgo* eggs, since the next generation could have an initial concentration originated by maternal transfer and the metals could be accumulated over generations, as reported with organic contaminants (Standley et al., 1994). The importance of this metal pollution in eggs in relationship to hatching success is not known, although comparative studies on the life cycle of this species in the studied area (Cid et al., 2008) did not detect large differences in its population abundances. This variability in metal dynamics described across the life cycle has important implications for the route of food chain transfer of metals in aquatic ecosystems, since not only the nymphs can carry the metals to higher trophic levels.

5. Concluding remarks

The high metal concentrations found in SPM and in the aquatic insects reflected the transport of Hg and Cd in the lower Ebro River to approximately 60 km downstream of the Flix chemical industry and suggested that a pathway of metal transfer via the ingested SPM can occur since SPM is the main food supply of these filter-feeders. While Hg pollution originated mainly at the chemical plant, the other metals analyzed showed upstream inputs from other parts of the basin. Interspecific differences in bioaccumulation were demonstrated by the higher metal concentrations bioaccumulated by *E. virgo* in comparison to *H. exocellata*., suggesting that fast feeding rate and poor metal elimination of *E. virgo* could be the main reasons. The analysis of up to 10 elements at the same time and analysis throughout the life cycle of an aquatic insect illustrates the high variability of metal dynamics, which could be related to the exposure route, the metal site of storage and the internal processes during development. With this in mind, further experimental studies under controlled conditions are required to more precisely describe the bioaccumulation patterns during growth. Nevertheless, this study confirms that evaluating metal presence at different life cycle stages demonstrates the increased complexity of pathways when analyzing the food chain transfer of metals in river ecosystems.

Acknowledgements

This study was funded by the Government of Catalonia (Departament d'Innovació, Universitats i Empresa i Departament de Medi Ambient i Habitatge), the European Social Fund and the Government of Spain (Ministerio de Educación y Ciencia, research project CGL2006-01487, Plan Nacional I+D+I; Ministerio de Medioambiente y Medio Rural y Marino; Consejo Superior de Investigaciones Científicas). We thank the Ebro Water Authority (Confederación Hidrográfica del Ebro) for the data on water chemistry, and the laboratory of the University of Barcelona, *Serveis Cientificotècnics*, for technical assistance in the metal analyses. We are very grateful to Carolina Solà (methods), Núria Bonada (Hydropsychidae taxonomy), Carles Alcaraz (statistics) and Rosa Andreu, David Mateu and Lluís Jornet (samplings). Also special thanks to Carolina Lourencetti and Joan Grimalt, who were present throughout the project.

References

Beauvais SL, Wiener JG, Atchison GJ. Cadmium and mercury in sediment and burrowing mayfly nymphs (*Hexagenia*) in the upper Mississippi River, USA. Arch Environ Contam Toxicol 1995; 28(2): 178-183.

Buchwalter DB, Cain DJ, Clements WH, Luoma SN. Using biodynammic models to reconcile differences between laboratory toxicity tests and field biomonitoring with aquatic insects. Environ Sci Technol 2007; 41(13): 4821-4828.

Buchwalter DB, Cain DJ, Martin CA, Xie L, Luoma SN, Garland T, Jr. Aquatic insect ecophysiological traits reveal phylogenetically based differences in dissolved cadmium susceptibility. Proc Natl Acad Sci USA 2008; 105(24): 8321-8326.

Cain DJ, Luoma SN, Carter JL, Fend SV. Aquatic insects as bioindicators of trace-element contamination in cobble-bottom rivers and streams. Can J Fish Aquat Sci 1992; 49(10):2141-2154.

Cain DJ, Carter JL, Fend SV, Luoma SN, Alpers CN, Taylor HE. Metal exposure in a benthic macroinvertebrate, *Hydropsyche californica*, related to mine drainage in the Sacramento River. Can J Fish Aquat Sci 2000; 57(2):380-390.

Cain DJ, Luoma SN, Wallace WG. Linking metal bioaccumulation of aquatic insects to their distribution patterns in a mining-impacted river. Environ Toxicol Chem 2004; 23(6): 1463-1473.

Carrasco L, Díez S, Soto DX, Catalán J, Bayona JM. Assessment of mercury and methylmercury pollution with zebra mussel (*Dreissena polymorpha*) in the Ebro River (NE Spain) impacted by industrial hazardous dumps. Sci Total Environ 2008; 407: 178-184.

Chapman PM, Wang FY, Janssen CR, Goulet RR, Kamunde CN. Conducting ecological risk assessments of inorganic metals and metalloids: current status. Hum Ecol Risk Asess 2003; 9(4): 641-697.

Cid N, Ibáñez C, Prat N. Life history and production of the burrowing mayfly *Ephoron virgo* (Olivier, 1791) (Ephemeroptera: Polymitarcyidae) in the lower Ebro River: A comparison after 18 years. Aquat Insects 2008; 30(3): 163-178.

Clements WH, Carlise DM, Lazorchak JM, Johnson PC. Heavy metals structure benthic communities in Colorado mountain streams. Ecol Apl 2000; 10 (2): 626-638

Currie RS, Fairchild WL, Muir DCG. Remobilization and export of cadmium from lake sediments by emerging insects. Environ Toxicol Chem 1997;16(11):2333-2338.

Day RW, Quinn GP. Comparisons of treatments after an analysis of variance in ecology. Ecol Monogr 1989;59(4):433-463.

De Haas EM, Reuvers B, Moermond CTA, Koelmans AA, Kraak MHS. Responses of benthic invertebrates to combined toxicant and food input in floodplain lake sediments. Environ Toxicol Chem 2002; 21(10): 2165-2171.

Dukerschein JT, Wiener JG, Rada RG, Steingraeber MT. Cadmium and mercury in emergent mayflies (*Hexagenia bilineata*) from the upper Mississippi River. Arch Environ Contam Toxicol 1992; 23(1): 109-116.

Evans RD, Balch GC, Evans HE, Welbourn PM. Simultaneous measurement of uptake and elimination of cadmium by caddisfly (Trichoptera : Hydropsychidae) larvae using stable isotope tracers. Environ Toxicol Chem 2002; 21(5): 1032-1039.

Goodyear KL, McNeill S. Bioaccumulation of heavy metals by aquatic macro-invertebrates of different feeding guilds: a review. Sci Total Environ 1999; 229(1-2): 1-19.

Grimalt JO, Sánchez-Cabeza JA, Palanques A, Catalán J. Estudi de la dinàmica dels compostos organoclorats persistents i altres contaminants en els sistemes aquàtics continentals. CSIC (Centro Superior de investigaciones Científicas), Barcelona, 2003.

Guillén J, Palanques A. Sediment dynamics and hydrodynamics in the lower course of a river highly regulated by dams: The Ebro River. Sedimentology, 1992; 39: 567-579.

Hare L, Saouter E, Campbell PGC, Tessier A, Ribeyre F, Boudou A. Dynamics of cadmium, lead, and zinc exchange between nymphs of the burrowing mayfly *Hexagenia rigida* (Ephemeroptera) and the Environment. Can J Fish Aquat Sci 1991; 48(1): 39-47.

Hare L. Aquatic insects and trace metals - bioavailability, bioaccumulation, and toxicity. Crit Rev Toxicol 1992; 22(5-6):327-369.

Ibáñez C, Escosa R, Muñoz I, Prat N. Life cycle and production of *Ephoron virgo* (Ephemeroptera: Polymitarcyidae) in the lower River Ebro (NE Spain). In: Alba-Tercedor J, Sánchez-Ortega, A., editors. Overview and strategies of Ephemeroptera and Plecoptera. The Sandhill Crane Press, Gainesville, Florida, U.S.A., 1991. pp. 483-492.

Ibáñez C, Pont D, Prat N. Characterization of the Ebre and Rhône estuaries: a basis for defining and classifying salt-wedge estuaries. Limnol Oceanogr 1997; 42(1): 89-101.

Inza B, Rouleau C, Tjalve H, Ribeyre F, Campbell PGC, Pelletier E, Boudou A. Fine-scale tissue distribution of cadmium, inorganic mercury, and methylmercury in nymphs of the burrowing mayfly *Hexagenia rigida* studied by whole-body autoradiography. Environ Res 2001; 85(3): 265-271.

Jop K, Wojtan K. Concentrations of cadmium and lead in the body of some macrobenthos species from five streams of Southern Poland. Acta Hydrobiol 1982; 24(3): 197-210.

Jop KM. Concentration of metals in various larval stages of four Ephemeroptera species. Bull Environ Contam Toxicol 1991; 46(6): 901-905.

Karouna-Renier NK, Sparling DW. Relationships between ambient geochemistry, watershed land-use and trace metal concentrations in aquatic invertebrates living in stormwater treatment ponds. Environ Pollut 2001;112(2):183-192.

Kureck A, Fontes RJ. The life cycle and emergence of *Ephoron virgo*, a large potamal mayfly that has returned to the River Rhine. Arch Hydrobiol Suppl 1996; 113(1-4): 319-323.

Lacorte S, Raldúa D, Martínez E, navarro A, Díez S, Bayona JM, Barceló, D. Pilot survey of a broad range of priority pollutants in sediment and fish from the Ebro river basin (NE Spain). Env Pollut 2006; 140: 471-482.

Linsinger T. Application Note 1: Comparison of a measurement result with the certified value. European Reference Materials. 2005; (2nd May 2008): http://www.erm-crm.org/html/ERM_products/application_notes/application_note_1/application_note_1_english_en.pdf

Luoma SN, Fisher N. Session 6. Critical issues in methodological uncertainty. In: Ingersoll CG, Dillon, T., Biddinger, G. R., editors. Ecological Risk Assessments of Contaminated Sediments. SETAC special publication series, Pensacola, Florida, U.S.A.,1997. pp. 211-237.

Luoma SN, Rainbow PS. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environ Sci Technol 2005; 39(7): 1921-1931.

McLachlan MS. Bioaccumulation of hydrophobic chemicals in agricultural food chains. Environ Sci Technol 1996; 30(1): 252-259.

Mañosa S, Mateo R, Guitart R. A review of the effects of agricultural and industrial contamination on the Ebro delta biota and wildlife. Environ Monit Assess 2001; 71(2): 187-205.

Maret TR, Cain DJ, MacCoy DE, Short, TM. Response of benthic invertebrate assemblages to metal exposure and bioaccumulation associated with hard-rock mining in northwestern streams, USA. J N Am Benthol Soc 2003; 22(4): 598-620.

Muñoz I, Prat N. Macroinvertebrate community in the lower Ebro River (NE Spain). Hydrobiologia 1994; 286 (2): 65-78.

Palanques A, Estrada F, Paracuellos B. Estudi geoquímic i sedimentològic dels fangs i contaminants sedimentats en el Riu Ebre a l'alçada de Flix. Informe tècnic.; 1996. 57 pp.

Palanques A, Plana F, Maldonado A. Recent influence of man on the Ebro margin sedimentation system, Northwestern Mediterranean. Mar Geol 1990; 95: 247-263.

Querol X, Alastuey A, López-Soler A, Mantilla E, Plana F. Mineralogy of atmosferic particulates around a large coal-fired power station. Atmos Environ 1996; 30(21): 3557-3572.

Ramos L, Fernández MA, González MJ, Hernández LM. Heavy metal pollution in water, sediments, and earthworms from the Ebro River, Spain. Bull Environ Contam Toxicol 1999; 63: 305-311.

Sánchez - Chardi A, López- Fuster MJ, Nadal J. Bioaccumulation of lead, mercury, and cadmium in the greater white-toothed shrew, *Crocidura russula*, from the Ebro Delta (NE Spain): Sex- and age- dependent variation. Environ Pollut 2007; 145: 7-14.

Saouter E, Hare L, Campbell PGC, Boudou A, Ribeyre F. Mercury accumulation in the burrowing mayfly *Hexagenia rigida* (Ephemeroptera) exposed to Ch_3HgCl or $HgCl_2$ in water and sediment. Water Res 1993; 27(6): 1041-1048.

Schofield RMS, Nesson MH, Richardson KA, Wyeth P. Zinc is incorporated into cuticular "tools" after ecdysis: the time course of the zinc distribution in "tools" and whole bodies of an ant and a scorpion. J Insect Physiol 2003; 49: 31-44.

Schuhmacher M, Domingo JL, Llobet JM, Corbella J. Evaluation of the effect of temperature, pH, and bioproduction on Hg concentration in sediments, water, molluscs and algae of the delta of the Ebro river. Sci Total Environ 1993; Suppl. Part 1: 117-125.

Smock LA. Relationships between metal concentrations and organism size in aquatic insects. Freshw Biol 1983; 13(4): 313-321.

Solà C, Burgos M, Plazuelo A, Toja J, Plans M, Prat N. Heavy metal bioaccumulation and macroinvertebrate community changes in a Mediterranean stream affected by acid mine drainage and an accidental spill (Guadiamar River, SW Spain). Sci Total Environ 2004; 333(1-3): 109-126.

SPSS Inc., 2008. SPSS® 16.0.2 Command Syntax Reference. SPSS Inc., Chicago.

Standley LJ, Sweeney BW, Funk DH. Maternal transfer of chlordane and its metabolites to the eggs of a stream mayfly *Centroptilum triangulifer*. Environ Sci Technol 1994;28: 2105-2111.

Tachet H. Ricoux P, Bournaud M, Usseglio- Polatera P. Invertebrés d'eau douce: systématique, biologie, écologie. CNRS Editions, Paris, 2000, 587 pp.

Terrado M, Barceló D, Tauler R. Identification and distribution of contamination sources in the Ebro basin by chemometrics modelling coupled to geographical information systems. Talanta 2006; 70(4):691-704.

Van Cappellen P., Wang Y. Cycling of iron and manganese in surface sediments; a general theory for the coupled transport and reaction of carbon, oxygen, nitrogen, sulfur, iron, and manganese. Am J Sci 1996; 296: 197-243.

Zizek S, Horvat M, Gibicar D, Fajon V, Toman MJ. Bioaccumulation of mercury in benthic communities of a river ecosystem affected by mercury mining. Sci Total Environ 2007; 377(1): 407-415.

Zhou QF, Zhang JB, Fu JJ, Shi JB, Jiang GB. Biomonitoring: An appealing tool for assessment of metal pollution in the aquatic ecosystem. Anal Chim Acta 2008;606(2):135-150

Annex 1. Water quality data (minimum and maximum values) of the sampled stations in the Ebro basin. Source: Water Authority: Confederación hidrográfica del Ebro (C. H. E.).

Station	**Flow** ($m^3 s^{-1}$)	**Conductivity** ($\mu S\ cm^{-1}$)	**pH**	**Temperature** (ºC)	**O_2** ($mg\ l^{-1}$)	**SS** ($mg\ l^{-1}$)	**$P\text{-}PO_4$** ($mg\ l^{-1}$)	**$N\text{-}NO_3$** ($mg\ l^{-1}$)	**$N\text{-}NH_4$** ($mg\ l^{-1}$)	**$S\text{-}SO_4$** ($mg\ l^{-1}$)
AB[1]	16.4- 81	516- 1000	7.80- 8.36	6.5- 26.4	4.3- 12.5	2- 21	<0.10- 0.72	7.1- 38.9	<0.10- 0.36	178.3- 280.7
MAT[2]	.	443- 497	8.4	9.1- 22.7	9.1- 12.4	<1- 7	<0.10	5.1- 9.7	<0.10	51.9- 59.3
MZ[3]	39.5- 840.6	768- 2400	7.7- 8.12	6.8- 24.3	6.2- 12.4	6- 131	<0.10- 0.42	12.3- 21.8	<0.10- 0.22	99.3- 510
A[4]	122- 1880	741- 1600	7.8- 8.4	8.1- 27.3	6- 10.8	<1- 44	<0.10- 0.31	5.9- 14.8	<0.10- 0.27	104.3- 365.8
M[5]	.	1082- 1611	8- 8.2	14.9- 23.9	8.1- 8.7	<1- 3	<0.10- 0.16	9.9- 13.3	<0.10	233.0- 359.2
T[6]	83.2- 1955.7	743- 1560	7.9- 8.6	9- 28.4	6.9- 11.5	<1- 23	<0.10- 0.54	4.6- 15	<0.10- 0.23	107.0- 352.4

[1] Data from 16/01/06 to 13/12/06, monthly measures. Corresponding to the CHE monitoring station Segre-Seròs.
[2] Data from 12/06/06 to 04/06/07, semestral period measures. Corresponding to the CHE monitoring station Matarranya- Mazaleón.
[3] Data from 17/01/06 to 06/09/07, monthly measures Corresponding to the CHE monitoring station Zaragoza-Almozara.
[4] Data from 04/01/06 to 25/09/07, weekly measures. Corresponding to the CHE monitoring station Ebro-Ascó.
[5] Data from 20/08/07 to 22/11/07, semestral period measures. Corresponding to the CHE monitoring station Ebro-Mora de Ebro.
[6] Data from 04/01/06 to 25/09/07, weekly measures. Referent to the C. H. E. station Ebro-Tortosa.

Annex 1. Continued.

Station	Hg ($\mu g\ l^{-1}$)	Cd ($\mu g\ l^{-1}$)	Ni ($\mu g\ l^{-1}$)	Cr ($\mu g\ l^{-1}$)	As ($\mu g\ l^{-1}$)	Pb ($\mu g\ l^{-1}$)	Cu ($\mu g\ l^{-1}$)	Ti ($\mu g\ l^{-1}$)	Zn ($\mu g\ l^{-1}$)	Mn ($\mu g\ l^{-1}$)
AB[1]	<0.1	<1	.	<2	2	<2		.	4	22
MAT[2]	<0.1	<1	.	<2	<2	<2	<2	.	16- 32	<2- 9
MZ[3]	<0.1	<1	.	<2	<2	<2- 4	<2- 11	.	3- 31	7- 43
A[4]	<0.05- <0.1	<1	<1- 6.6	<1- <5	<1- 6.7		<2- 12	.	<2- 68	5- 41
M[5]	.	.	.	.	.	.	.	.	.	.
T[6]	<0.05-<0.1	<1	<1- 7	<1- <5	<1- 5.4	<2- <3	<2- 32	.	<2- 217	<7- 41

[1] Data from 16/01/06 to 13/12/06, monthly measures. Corresponding to the CHE monitoring station Segre-Seròs.
[2] Data from 12/06/06 to 04/06/07, semestral period measures. Corresponding to the CHE monitoring station Matarranya- Mazaleón.
[3] Data from 17/01/06 to 06/09/07, monthly measures Corresponding to the CHE monitoring station Zaragoza-Almozara.
[4] Data from 04/01/06 to 25/09/07, weekly measures. Corresponding to the CHE monitoring station Ebro-Ascó.
[5] Data from 20/08/07 to 22/11/07, semestral period measures. Corresponding to the CHE monitoring station Ebro-Mora de Ebro.
[6] Data from 04/01/06 to 25/09/07, weekly measures. Referent to the C. H. E. station Ebro-Tortosa.

Annex 2. Mean concentration ($\mu g \cdot g^{-1}$ DW) and standard error (in brackets) in *Ephoron virgo* larvae at different stations.

Station	N	Hg	Cd	Ni	Cr	As	Pb	Cu	Ti	Zn	Mn
MZ	6	0.06	0.55	7.67	12.77	7.23	5.52	20.61	209.67	203.87	131.60
		(0.01)	(0.12)	(0.90)	(1.60)	(0.35)	(0.82)	(0.90)	(37.17)	(8.19)	(17.14)
T	12	0.65	2.18	8.66	13.36	7.46	9.98	21.28	187.16	261.41	343.63
		(0.03)	(0.17)	(0.46)	(0.79)	(0.34)	(1.83)	(0.37)	(22.37)	(8.98)	(21.62)

Annex 3. Mean concentration (µg· g^{-1} DW) and standard error (in brackets) in *Hydropsyche* larvae at different stations.

Station	Description	N	Hg	Cd	Ni	Cr	As	Pb	Cu	Ti	Zn	Mn
AB	Control	3	0.03	0.06	3.39	3.96	2.05	4.64	22.20	51.67	143.27	314.18
			(0.01)	(0.01)	(0.73)	(1.22)	(0.22)	(2.50)	(0.94)	(16.65)	(4.73)	(30.05)
MAT	Control	6	0.04	0.03	1.00	2.33	2.16	1.08	18.34	19.02	118.50	40.53
			(0.00)	(0.00)	(0.09)	(0.09)	(0.22)	(0.19)	(1.18)	(2.30)	(4.87)	(6.81)
MZ	Control	3	0.04	0.13	4.69	5.99	2.06	3.65	16.15	69.59	132.78	92.23
			(0.00)	(0.01)	(0.77)	(1.31)	(0.31)	(1.08)	(0.75)	(32.34)	(8.09)	(11.37)
A	8 Km	6	0.93	0.15	5.55	3.67	2.43	1.83	17.28	47.39	144.10	633.03
			(0.38)	(0.02)	(0.41)	(0.30)	(0.11)	(0.19)	(1.17)	(7.22)	(4.41)	(186.91)
M	21 Km	3	0.46	0.32	6.44	5.85	2.96	4.55	16.87	90.04	163.74	529.73
			(0.03)	(0.02)	(0.80)	(0.48)	(0.32)	(0.55)	(0.28)	(14.26)	(6.69)	(29.35)
T	68 Km	3	0.29	0.13	3.39	4.78	2.01	2.94	17.42	43.71	137.48	324.06
			(0.04)	(0.02)	(0.88)	(1.21)	(0.31)	(0.78)	(1.27)	(10.57)	(4.70)	(41.90)

Annex 4. Mean concentration (µg· g^{-1} DW) and standard error (in brackets) in T during 2007 sampling period for the two taxons analyzed.

Species	N	Hg	Cd	Ni	Cr	As	Pb	Cu	Ti	Zn	Mn
Ephoron virgo	6	0.64	1.77	8.41	14.08	6.47	11.79	20.30	217.20	241.99	308.70
		(0.05)	(0.20)	(0.77)	(0.95)	(0.28)	(3.60)	(0.34)	(31.08)	(12.63)	(14.03)
Hydropsyche exocellata	3	0.29	0.13	3.39	4.78	2.01	2.94	17.42	43.71	137.48	324.06
		(0.04)	(0.02)	(0.88)	(1.21)	(0.31)	(0.78)	(1.27)	(10.57)	(4.70)	(41.90)

Annex 5. Mean concentration (µg· g^{-1} DW) and standard error (in brackets) in *Ephoron virgo* larvae along larval growth for 2005, 2006 and 2007. Body length (BL, in mm) values were extracted from Cid et al. (2008). N is number of replicates containing several individuals in each replicate.

Date	**BL**	**N**	**Hg**	**Cd**	**Ni**	**Cr**	**As**	**Pb**	**Cu**	**Ti**	**Zn**	**Mn**
29-May-05	6.4	3	0.59	1.55	.	10.96	7.57	9.48	21.43	61.32	224.23	438.10
	(1.6)		(0.16)	(0.43)	.	(4.22)	(2.39)	(3.51)	(6.50)	(17.21)	(59.44)	(162.36)
12-Jun-05	7.5	3	0.65	1.43	.	5.70	4.58	5.42	24.66	28.17	182.69	228.36
	(2.1)		(0.22)	(0.29)	.	(2.14)	(1.87)	(2.46)	(6.33)	(10.32)	(20.49)	(108.33)
04-Jul-05	10.9	3	0.93	1.98	.	6.67	6.92	6.99	24.32	28.67	230.46	273.51
	(3.5)		(0.06)	(0.14)	.	(0.49)	(1.01)	(0.67)	(2.62)	(2.12)	(7.39)	(20.44)
04-Aug-05	15.7	3	0.47	1.54	.	4.48	5.16	4.62	21.89	19.32	240.48	268.73
	(2.6)		(0.16)	(0.29)	.	(0.82)	(1.41)	(0.56)	(2.01)	(2.49)	(21.94)	(54.21)
14-Jun-06	10.6	3	0.62	2.62	10.08	15.51	8.17	9.68	21.92	212.58	280.80	458.10
	(2.1)		(0.09)	(0.28)	(0.04)	(0.09)	(0.21)	(0.13)	(0.25)	(30.06)	(10.71)	(4.35)
29-Jun-06	13.2	3	0.71	2.56	7.72	9.77	8.74	6.65	22.62	101.67	280.85	299.01
	(2.1)		(0.04)	(0.17)	(0.27)	(0.26)	(0.14)	(0.45)	(0.47)	(19.82)	(10.41)	(22.60)
05-Jun-07	.	3	0.55	1.35	8.20	16.09	6.58	16.70	19.89	265.05	214.26	335.62
	.		(0.06)	(0.11)	(0.51)	(0.58)	(0.45)	(6.37)	(0.60)	(48.78)	(3.60)	(10.42)
20-Jun-07	.	3	0.73	2.18	8.63	12.08	6.36	6.89	20.70	169.35	269.72	281.78
	.		(0.01)	(0.11)	(1.64)	(0.40)	(0.42)	(0.27)	(0.26)	(12.66)	(3.85)	(12.31)

Annex 6. Mean concentration (µg· g^{-1} DW) and standard error (in brackets) in *Ephoron virgo* along different life cycle stages for 2005, 2006 and 2007 (S.E.).

Life cycle	**N**	**Hg**	**Cd**	**Ni**	**Cr**	**As**	**Pb**	**Cu**	**Ti**	**Zn**	**Mn**
Nymphs	24	0.66	1.90	8.66	10.16	6.76	8.30	22.18	110.76	240.44	322.90
		(0.04)	(0.12)	(0.46)	(0.99)	(0.46)	(1.10)	(1.06)	(19.58)	(9.89)	(26.86)
Eggs	6	0.29	0.01	3.15	1.37	0.24	0.42	5.31	1.71	75.73	15.60
		(0.04)	(0.00)	(1.33)	(0.08)	(0.02)	(0.17)	(0.61)	(0.31)	(1.40)	(0.72)
Male moult	3	0.15	0.09	1.17	4.73	0.82	20.47	51.62	12.01	47.75	23.46
		(0.07)	(0.05)	(0.26)	(0.24)	(0.05)	(5.57)	(11.57)	(2.67)	(6.21)	(4.99)
Females	15	0.58	0.63	0.28	1.72	0.63	2.21	23.14	5.10	94.36	27.03
		(0.05)	(0.07)	(0.07)	(0.23)	(0.25)	(0.61)	(1.53)	(1.43)	(4.07)	(11.74)
Males	6	0.64	1.34	0.29	1.82	0.58	1.72	45.36	4.79	66.10	4.00
		(0.10)	(0.23)	(0.03)	(0.52)	(0.09)	(0.63)	(5.21)	(1.28)	(7.05)	(0.75)

Chapter 5

Organochlorine bioaccumulation in the filter-feeding mayfly *Ephoron virgo* during life cycle in a site with chronic pollution

Cid, N., Lourencetti, C., Ibánez, C., Prat, N. & Grimalt, J. O.

***Environmental Science and Technology* (to be submitted).**

Organochlorine bioaccumulation in the filter-feeding mayfly *Ephoron virgo* during life cycle in a site with chronic pollution

NÚRIA CID [*§‡], CAROLINA LOURENCETTI [†], CARLES IBÁÑEZ [‡], NARCÍS PRAT [§], AND JOAN O. GRIMALT [†]

Department of Ecology, University of Barcelona, Diagonal 645, 08028 Barcelona, Catalonia, Spain, IRTA, Aquatic Ecosystems Unit, Sant Carles de la Ràpita, Catalonia, Spain, Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Jordi Girona, 18, 08034 Barcelona, Catalonia, Spain.

Abstract

The fate of organochlorine compounds (OCs), penta- and hexa-chlorobenzene, (PeCB and HCB) hexachlorociclohexanes (HCHs), dichlorodiphenyl-trichloroethanes (DDTs) and polichlorobophenyls (PCBs), were evaluated across the different life cycle stages of the mayfly *Ephoron virgo* in a wild population from a site with chronic pollution for these compounds due to the presence of a sediment dump upstream. This mayfly was selected because of its high sensitivity to pollutants, its ecological relevance, and its bioaccumulation potential as a filter-feeder. The pollution bioaccumulation was assessed by contrasting OC levels downstream of the pollution source with an upstream site used as control. Increasing values of HCB, HCHs, DDEs and PCBs with nymph size was observed, evidencing bioconcentration during growth. Emergent imagoes and subimagoes presented the highest OC levels, with increases ranging from 2 to 8- fold the value of nymphs. This may have important implications in the transfer to higher trophic levels, since these stages can be easy food source for predators. Sexual differences in bioaccumulation were observed since male imagoes had higher DDEs and PCBs than subimago females. However, and as a result of a low maternal transfer, eggs presented the lowest concentrations containing up to 5 times less the value of mothers. The results denoted a life cycle-related variability in OC bioaccumulation in this species, which should be considered when evaluating contaminant transfer in aquatic and riparian food webs, and when linking the results of ecotoxicological laboratory studies with those in natural habitat conditions. In general, the OC load detected in this keystone species evidenced the ecological risk in the lower part of the Ebro River for aquatic communities, mainly originated at the large sediment dump located in the area.

[§] Department of Ecology, Faculty of Biology, University of Barcelona, Catalonia, Spain
[‡] IRTA, Aquatic Ecosystem Unit , carretera Poble Nou km 5.5, 43540 St. Carles de la Ràpita, Catalonia, Spain.
[†] Department of Environmental Chemistry (IDÆA-CSIC)
[*] Corresponding author phone: 34 977 74 54 27 ext. 1820; fax: 34 977 74 41 38; e-mail: nuria.cid@irta.cat

Introduction

Nowadays many rivers in different countries suffer processes of contamination, such as point-source occasional or continuous release of pollutants (Holt, 2000; Malmqvist & Rundle, 2002). Several potentially toxic contaminants as the organochlorine compounds (OCs) readily adsorb onto fine-grained organic and inorganic particles, which can be transported long distances before being deposited in areas of low current velocity and ingested by filter-feeding organisms (Steingraeber & Wiener, 1995). Moreover, these compounds, such as penta- and hexa-chlorobenzene, (PeCB and HCB) hexachlorociclohexanes (HCHs), dichlorodiphenyl-trichloroethanes (DDTs) and polichlorobophenyls (PCBs), present high persistence in the environment and thereby an elevated potential for bioaccumulation in aquatic food webs (Kidd et al., 1998).

Benthic aquatic insects can accumulate and transfer these contaminants through riverine ecosystems since they can process the organic matter and are an important component of the diet to higher trophic levels. They have been used as sentinels of environmental pollution (Standley & Sweeney, 1995; Corkum et al., 1997) and as a source for identification of contaminant biomagnification (Morrissey et al., 2005; Vives et al., 2005a). However, the bioaccumulation of pollutants in aquatic insects can change with size and life cycle stages (Smock, 1983; Cain et al., 1992; Standley et al., 1994; Bartrons et al., 2007) since the type and amount of contaminants incorporated can be influenced by the life history and feeding habits of the organism.

The patterns of OC concentration along development and life stages of insects are still not well known and there is a need to better understand their ecological implications. The present study aims to analyze the OCs transfer along all the life cycle stages of the aquatic insect *Ephoron virgo* (Olivier) (Ephemeroptera: Polymitarcyidae). This mayfly was thought to be the appropriate study organism due to its ecological traits and role into the food web. *E. virgo* nymphs are benthic particle-feeders which build a U-shaped tube and feed on particles of organic matter from water (Kureck & Fontes, 1996) which can be bound to contaminants. Its univoltine life cycle gives the adequate conditions to study OC patterns in its natural habitat since nymphs hatch in April, and grow rapidly within a period of 3 to 4 months, followed by emergence and reproduction, when eggs are deposited in the river and spend the autumn and winter in a diapauses attached to river sediment. *E. virgo* contributes with a large biomass to the food web, with

estimates of annual nymph production of 950 mg/m^2/year dry weight and adult mass emergences in the lower Ebro River (Cid et al., 2008) and is an abundant prey for fish and birds (van der Geest et al., 2000). Moreover, this species is appointed as a good bioindicator of ecological quality sine is sensitive to organic pollution and sediment-bound toxicants (De Haas et al., 2002), thus is of special concern in the protection of river ecosystems in its area of distribution.

With all this in mind, the bioaccumulation patterns of OCs along the nymph growth and the transfer from nymphs to adults, and from females to eggs, were studied over two life cycles (2005 and 2007) in a wild population from the lower Ebro River which is under the impact of chronic industrial pollution. Moreover, *E. virgo* concentrations in larvae at this site were compared with populations upstream of the impacted area in order to quantify the bioaccumulation attributed to this point source of organochlorine pollution. Thus, the main objectives of the present study were to: (1) assess the impact of pollution by its bioaccumulation on keystone species such as *E. virgo*, (2) evaluate the bioaccumulation process during the nymph growth of this species and during all life cycle stages.

Materials and Methods

Study Site and Sampling

The study area was located in the Ebro River (NE Iberian Peninsula, Spain). The Ebro River has a drainage basin of 85,550 km2 with a length of 928 km and annual average flow of 426 m3·s-1. The Ebro basin is impacted by industrial, urban and agricultural activities and the presence of organic compounds and metals have been reported in many sites along the river (Fernández et al., 1999; Lacorte et al., 2006; Terrado et al., 2006; Bosch et al., 2009). The lower part of the river receives a potential influx of OCs originated from a chemical plant located in the town of Flix (100 km from river mouth). Here, more than 300,000 t of polluted industrial solid wastes from alkali-chlorine electrolysis, phosphate fertilizer and pesticide production have been deposited since the completion of the Flix dam in 1949, and until the 1980s when the deposition of solid wastes into the reservoir was banned. Pollutants present in the solid waste of the factory include organochlorine compounds (hexachlorobenzene, polyclhorostyrenes, polychloronaphtalenes, PCBs and DDTs) and heavy metals (mercury, cadmium, chromium and nickel) and have been detected in the sediments at very high

concentrations (Grimalt, 2006). Since OCs are very insoluble in water but can be easily adsorbed to fine sediments, they can be released downstream with the suspended particulate matter (Gómez-Gutiérrez et al., 2006).

In order to evaluate the impact of the organochlorine pollution from the sediment dump (first objective), *E. virgo* nymph samples were taken 68 km downstream of the pollution source (upstream of the city of Tortosa, Station T: 40° 49' 27.22" N, 0° 31' 6.03" E) and 250 km upstream of the pollution source (upstream of the city of Zaragoza, at the town of Monzalbarba, Station MZ: 41° 42' 35.48" N, 0° 57' 33.71" W) during late spring of 2006 and 2007 (see Cid et al., 2010, for details of site locations and heavy metal bioaccumulation in the area). located upstream of the Flix dam and of the main reservoirs in the lower course of the Ebro River. These two locations are areas where populations are known to be abundant (Torralba-Burial & Ocharan, 2004; Cid el al., 2008) and contained a sufficiently large population of *E. virgo* for larval sample comparisons, while in other areas of the river they are inexistent or abundances are very low mainly due to the presence of reservoirs (habitat loss) or other anthropogenic impacts as pollution. At Station T, the characteristics of the life cycle and secondary production of *E. virgo* are well known (Ibáñez et al., 1991; Cid et al., 2008). The univoltine life cycle and the synchronized massive adult emergences allowed the collection of different nymph instars, adult males, subimago females and eggs in its natural habitat. Thus, for the second objective , different instars of the nymphs were collected in late May, June, July and August during 2005, and only two instars in June during 2007. At the field, nymphs were sampled using a kick net (250 μm mesh net). The riverbed was disturbed and the subsequent sample was deposited in a tray to identify and collect the individuals in situ. Organisms were cleaned with double distilled water, placed in aluminum paper envelopes and stored with ice during transport. Data on nymph body length for 2005 were obtained from Cid et al. (2008), corresponding to samples taken in the same study site. When adult emergences began in early August (2005 and 2007), adults were sampled by light trapping. Adult females (subimago), adult males (imago) and eggs extracted from subimago females were collected separately. Samples were composed by three replicate samples containing approximately 7-8 individuals per replicate. Samples were kept frozen at -20ºC until analysis.

Chemicals

Standards of the following analyzed compounds were used: Pesticide Mix 164 (DDDs, DDEs, DDTs), Pesticide Mix 11 (HCHs, hexachlorobenzene) and pentaclhorobenzece in cyclohexane, and PCB Mix 11 (PCB#28, PCB#52, PCB#101, PCB#118, PCB#138, PCB#153, PCB#180), PCB#30, PCB#200 and PCB#142 in isooctane, with purity higher than 97%. They were purchased from Dr. Ehrenstorfer (Augsburg, Germany). All the solvents, n-hexane, dichloromethane, isooctane, were residue analysis grade and from Merck (Darmstadt, Germany).

Organochlorine Compound Analysis and Quality Assurance

The analytical method for OCs analysis is described in detail elsewhere (Vives et al., 2002; Bartrons et al., 2007). After drying the samples in a vacuum-sealed drier at 20 ºC until constant weight, approximately 0.02 - 0.09 g of the samples were spiked with surrogates, PCB#30 and PCB#200 standards. Organochlorine compound extraction was performed by sonication with four successive steps of 15 min with hexane/dichloromethane (4:1). Clean-up was performed with oxidation (concentrated sulfuric acid – 2 mL at a time) and shaking until obtaining a final clean organic extract. The resulted organic-phase was concentrated to near dryness under a gentle flow of nitrogen and redissolved in 100 μL of standard internal solution, PCB#142.

The identification and quantification of the OCs in the samples were carried out by gas chromatography coupled to electron capture detection (GC-ECD, Hewlett-Packard 5890 series II) with a 60 m x 0.25mm i.d. DB-5 capillary column (J&W Scientific, Folsom, CA) coated with 5% phenyl/95%methylpolysiloxane (film thickness 0.25 μm). The GC operated in splitless mode. The oven temperature program started at 90ºC (held for 2 min), ramped to 150ºC at 15 ºC·min-1 and then to 290 ºC at 4 ºC·min-1 (holding time 20 min). Injector and detector temperatures were 280 and 310 ºC, respectively. Helium and nitrogen were used as carrier (1.5 mL·min-1) and make up (60 mL·min-1) gases, respectively. Gas chromatography (HP 5973 MSD) coupled to mass spectrometry operating in a negative ion chemical ionization (GC-MS-NICI) (Agilent, Palo Alto, USA) was employed for analyte confirmation. Samples were injected in splitless mode onto a similar column used for GC-ECD analysis. Helium was used as carrier gas (1.0 mL·min-1). Ammonia was used as reagent gas (1.75 mL·min-1). The temperature

program started at 90 ºC (held for 2min), then increased to 150 ºC at 10 ºC min-1 and to 310 ºC at 4 ºC·min-1 with a final holding time of 20min. Injector, ion source and transfer line temperatures were 250, 176 and 280 ºC, respectively. The dwell time was 50 ms channel-1. Confirmation ions were m/z 221 [PCB#28]-1; 250 [PeCB]-1; 225 [2,4'-DDT]-1; 246 [2,4'-DDE]-1; 255 [HCHs]-1; 281 [4,4'-DDE]-1; 283 [4,4'-DDT]-1; 284 [HCB]-1; 291 [PCB#52]-1; 320 [2,4'-DDD]-1 and [4,4'-DDD]-1; 291 and 326 [PCB#101]-1, [PCB#118]-1; 326, 360 [PCB#138]-1, [PCB#153]-1; 360, 394 [PCB#180]-1.

Quantification was performed by internal standard mode. The recoveries of the surrogates, PCB#30 and PCB#200, were calculated for each sample, being 53 ± 16 % and 74 ± 12 %, respectively. Surrogate recoveries as well as procedure blanks were used to correct the concentration of the analytes. Limits of detection (LOD) and quantification (LOQ) were calculated from blanks by averaging the signal of all blanks plus 3 and 10 times the standard deviation, respectively. For compounds absent from the blanks, the limits of detection were calculated from the background signals of the instrument using injections of diluted standards. The LOD of individual congeners varied between 0.019 to 3.9 ng·g-1.

Statistical Analysis

In samples where organochlorine compound concentration was lower than the detection limit, values used for statistical analyses were half the value of the detection limit (Karouna-Renier & Sparling, 2001). In order to test for differences in OCs burdens among sites and life cycle stages of *E. virgo* an analysis of variance (ANOVA) was performed, followed by a Games-Howell post-hoc test. Games-Howell tests are post-hoc multiple comparison tests that are among the most powerful and robust for unequal variances (Day & Quinn, 1989). In order to test if concentration changes of OC compounds showed any relationship with nymph development, the significant variation among different nymph sizes was decomposed in a residual component with polynomial orthogonal contrasts (Sokal & Rohlf, 1995).Variables were log transformed for parametric analyses because homoscedasticity and linearity were clearly improved. Statistical analyses were performed with SPSS 16.0.2

Results and Discussion

Differences in Organochlorine Concentrations among sites

Because no significant differences in OC values for nymphs were obtained for the two sampled years (2006 and 2007) among sampling sites, we did not consider the interannual variation for our analysis. Mean concentrations in *E. virgo* showed a significant increase of all OCs analysed at site T downstream of the sediment dump (Figure 1), except for γ-HCH which values were higher upstream at site MZ, upstream of the sediment dump. Neither α-HCH nor β-HCH nor δ-HCH was detected in this organism at any site. The highest concentrations were obtained for 4,4'-DDE, 4,4'-DDT, PCB-153, PCB-138 and PCB-180 (mean values from 62 to 187 ng·g-1 dw). However, the most significant increases were observed for 2,4'-DDE, 2,4'-DDT, 4,4'-DDT and PCB-52, with values 35, 33, 24 and 70-fold higher at site T than at site MZ, respectively. For the other OC compounds values at T ranged from 4 to 15 times higher.

The compounds showing the highest concentrations were 4,4'-DDE, 4,4'-DDT and the PCBs congeners. Among the PCBs analyzed, PCB#138, PCB#153, PCB#180 were the predominant compounds and those presenting a higher K_{ow} (octanol water partition coefficient) than the others analyzed, which could result in a higher biocumulation in the studied organism (MedChem, 1996). Besides, Koc was also higher than the other compounds, and therefore could be easily associated to the sediment particles. Moreover, these OCs compounds have been identified in the sediment dump located 68 Km upstream the sampling site and also in the particulated and dissolved phases of water samples and in sediment samples from the Ebro River (Grimalt, 2006; Lacorte et al., 2006; Gómez-Gutiérrez et al., 2006; Bosch et al., 2009). Thus, these compunds can be bioaccumulated at high concentrations in the organisms due to a potentially high exposure. The OC levels obtained in this aquatic insect (with the exception of HCHs) at station T downstream of the pollution source are much higher than those reported in high mountain lakes (Campbell et al., 2000; Catalán et al., 2004; Bartrons et al., 2007) where OCs have mainly an atmospheric source. As far as we know, no comparable OC data exist in aquatic insects from other rivers with a high pollution load, only one reporting PCBs in emergent mayflies in the upper Mississipi River (Steingraeber et al., 1994). The levels of PeCB, HCB, DDTs and breakdown products and PCBs in benthic macroinvertebrates from the lower Ebro are the highest ever reported in aquatic

ecosystems, warning about the high levels of bioavailable hazardous chemicals in this area mainly due to chronic pollution.

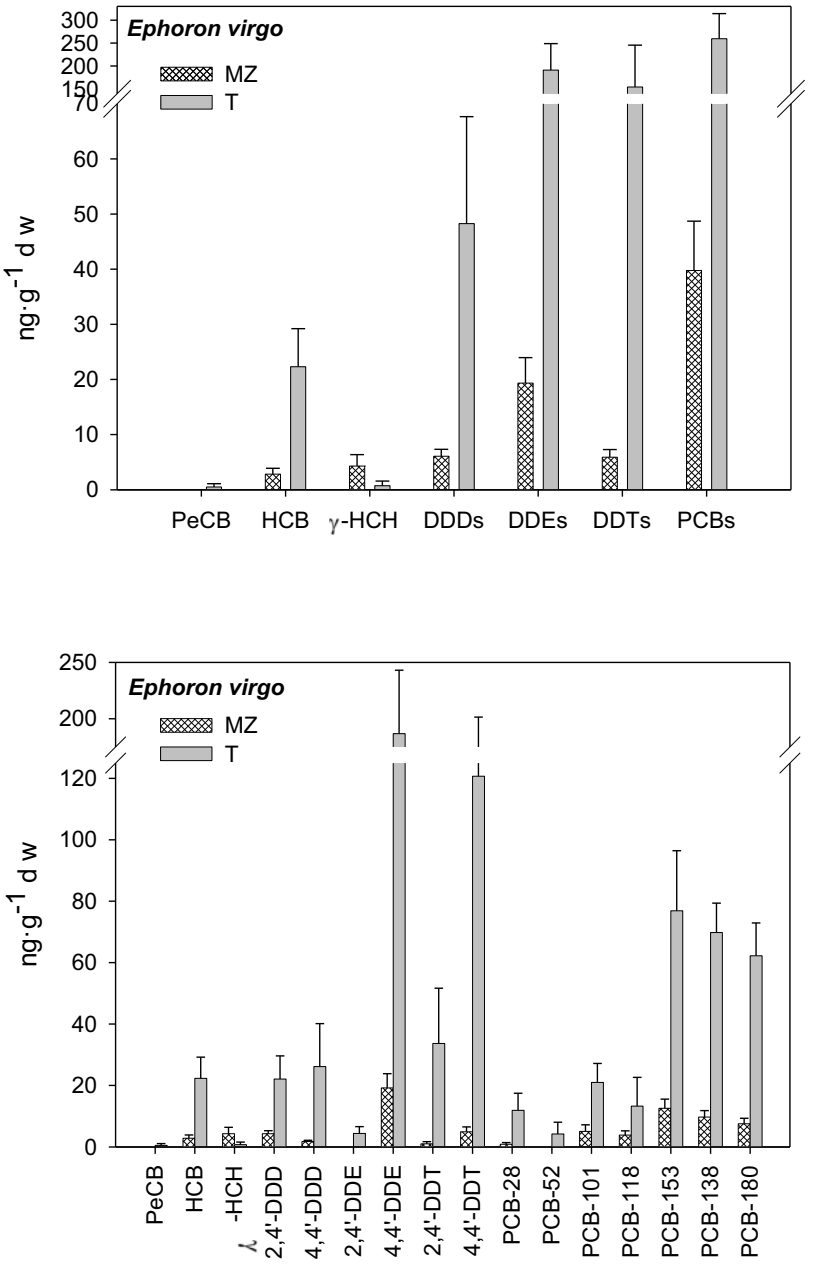


FIGURE 1. Concentrations (ng·g^{-1} dw) of organochlorine compounds detected in nymphs of *E. virgo* from the study area. MZ is the site located upstream the sediment dump and T is downstream. All OC concentrations are significant (P<0.05), except for γ-HCH.

The mayfly *E. virgo* is appointed as a test organism due to its sensitivity to pollutants, which may play a key role in assessing the ecological status of rivers in Western Europe that suffer from pollution with a wide variety of toxicants (Van der Geest et al., 2000; De Haas et al., 2002). Thus, it is important to note that T site is located 68 km

downstream of the waste dumping site and that was the first area where populations of *E. virgo* were abundant. In addition, a personal observation of the behavior *E. virgo* nymphs when they were placed in trays at the field was that at site MZ (upstream) they exhibited a very active behavior with a very fast movement of the body and the tracheal gills, while at site T nymphs were moving slowly and presented a weak appearance. Despite many abiotic and biotic factors can determine the presence of species in highly polluted rivers, as habitat constraints, food availability and its sensitivity to contaminants (De Haas & Kraak, 2008), the high pollution load downstream of the pollution source is hypothesized to be one of the main causes explaining *E. virgo* absence or low abundances at the first 50 km after the sediment dump. Recent studies in the area affected by the Flix sediment dump reported dioxin-like toxic effects in cell line activities and in fish (Eljarrat et al., 2008; Bosch et al., 2009), as well as alterations in some bioachemical markers (Lavado et al., 2006) associated to the high levels of PCBs in the environment. Experiments with particle-feeders as *Daphnia magna* have shown a feeding depression after exposures to pesticide mixtures (Barata et al., 2008) and sublethal effects affecting the life cycle have also been detected in tolerant species as the midge *Chironomus riparius* exposed to polycyclic aromatic compounds and pesticides (Hahn et al., 2002; Paumen et al., 2008). Other studies focusing on macroinvertebrates showed reduced secondary production and malformations in highly polluted environments (Edsall et al., 1991; Bonada et al., 2005; Skinner & Bennett, 2007). Therefore, even though Station T appeared to be the first area downstream of the sediment dump where *E. virgo* population were abundant, this species could suffer sublethal toxicity effects due to the high OC bioaccumulation.

Organochlorine Concentrations during *Ephoron virgo* Nymph Growth

The largest concentration changes were detected for HCB, ∑DDEs and ∑PCBs, which showed a slight decrease from first to second instars, followed by a steady increase until late instars. The concentration change in the latter compounds was significant when comparing nymphs with a mean body length of 7.5 mm to those about to emerge (Table 1). γ-HCH was not detected in the first instars but was present in the last two, observing the highest concentrations in latter nymphs. When performing the polynomial contrasts, HCB, γ-HCH, ∑DDE and ∑PCB concentrations in 2005 showed a significant linear

relationship with nymph size (Table SI2), describing a general pattern of OC bioconcentration with nymph growth. The more representative bioaccumulated DDE congener increasing with nymph growth was 4,4'-DDE, while for PCBs it was PCB#153, exhibiting concentration changes about 2 and 1 fold higher, respectively, between each instar (Table SI1); and also following a linear relationship according to general ∑DDE and ∑PCB patterns. PCB#52 and 2,4'-DDE were only detected in one instar and at very low levels.

TABLE 1. Concentration of Organochlorine Compounds in *Ephoron virgo* nymphs during 2005.

Date	Mean size[1] (mm)± SD	Mean concentration (ng g^{-1} dw)[2] ± SD HCB	γ-HCH	∑DDD	∑DDE	∑DDT	∑PCB
29-May-05	6.4±1.6	18 ± 1ab	ND	74±14a	141 ± 12a	148 ± 41a	134 ± 4ab
12-Jun-05	7.5±2.1	21 ± 3a	ND	32±6a	80 ± 2a	62 ± 7a	91 ± 5a
04-Jul-05	10.9±3.5	52 ± 4ab	8 ± 7a	42±7a	286 ± 58ab	107.5 ± 0.2a	163 ± 8ab
04-Aug-05	15.7±2.6	83 ± 13b	28 ± 16a	72±2a	448 ± 28b	142 ± 6a	241 ± 15b

[1] body length from Cid et al. (2008). [2] replicates containing several individual in each one (n = 3). ND: not detected. Results in the same column sharing the same letter are not significantly different ($P < 0.05$)

In contrast, neither PeCB nor α, ß, δ-HCH were detected, and no statistical differences were observed for ∑DDT and ∑DDD among nymph sizes. Although no significantly differences were detected, ∑DDD (including 2,4'-DDD and 4,4'-DDD) and the congener 2,4'-DDT presented a similar pattern in nymphs. That is, the first instar showed similar concentrations for these compounds than the last one, but observing a decrease in the second and third instars with values half those at the beginning and at the end of growth.

The amount of OCs incorporated by growing nymphs depends on its bioavailability in the river and therefore on its dilution and dispersion downstream. No information is available on the main uptake routes of pollutants in *E. virgo*, but since the industrial sediment dump located upstream of Station T contains large amounts of hazardous materials (Grimalt et al., 2006), the particle-bound OCs released downstream can be the main uptake source into the organisms via ingestion. Thus, OCs circulating in the river bound to particulate matter throughout the year, as PCBs and DDTs, (Gómez-Gutiérrez

et al., 2006) are likely uptaken as food and can bioconcentrate as nymphs grow, mainly in late instars. This pattern is described in other Ephemeroptera as *Hexagenia limbata* which bioconcentration occurred as a result of ingestion of contaminants sorbed to sediment organic carbon (Drouillard et al., 1996). The general pattern of these compounds to increase with nymphal development may be also related to a change in lipid content, having the older nymphs the highest lipid stores, according to the energy available in the stage prior to emergence and reproduction, as seen in other Ephemeroptera (Meyer, 1990; Cavaletto et al., 2003). This suggests that OCs may follow the patterns of lipid storing along growing nymphs. On the other hand, the general decrease of OCs from the first to second instar could be explained by a shift in diet since early instars feed on fine particulate organic matter and latter instars can also feed on suspended detritus and algae (De Lange et al., 2005). Therefore, the particle size and nature of ingested food at each instar could also be a factor to take into account to explain the patterns of OC bioaccumulation.

Considering that the studied species is heterometabolous and moults along its growth, we presume that some amount of OCs could be adsorbed to the exoskeleton and that the moulting process could be involved in their elimination, as observed for some heavy metals in this species (Cid et al., 2010). No previous studies on aquatic insects have reported OC concentrations according to the nymph or larval growth, and neither the biotransformation of these products by insects. The high presence of DDT breakdown products suggest that they could be originated from the biotransformation of DDT along the mayfly growth, since organisms can biotransform DDT, i. e. transforming 4,4'-DDT to 4,4'-DDE (Vives et al., 2005b). However, this fact was not observed in our study as the ratio between 4,4'-DDT and 4,4'-DDE over the life cycle did not present any significant variation (0.336 ± 0.13, average ± standard deviation) suggesting the 4,4'-DDE might be mainly uptaken directly from the environment.

Organochlorine transfer across life cycle stages

When measuring the different life cycle stages of *E. virgo* over two years, 2005 and 2007, significant interannual differences were observed(P<0.05). Despite these differences, similar trends of bioaccumulation were observed (Figure 2). For both studied years, adults (mainly males) presented the highest concentration for the total sum of OCs (Table SI3). Eggs clearly showed significantly lower values compared with

adults ($p<0.05$), except for PeCB, because some samples were below the detection limit or were at very low concentrations. PCBs and DDDs were present in eggs at significantly lower concentrations than in nymphs and adults ($p<0.05$) while DDTs, DDEs, HCHs and HCB had similar values than nymphs but lower values than adults.

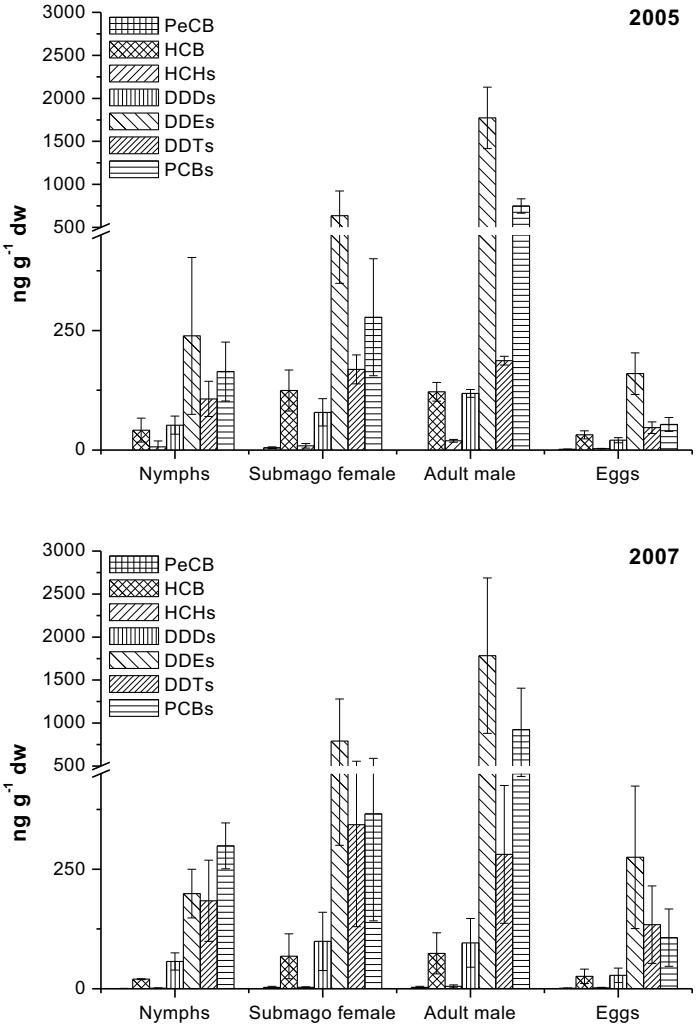


FIGURE 2. Concentration of organochlorine compounds over different stages of development of *Ephoron virgo* in 2005 and 2007.

The OCs transfer from subimago females to eggs in this study was very low (Figure 3), with a mean ratio of 0.3 and 0.4 for 2005 and 2007, respectively. The high OC concentrations found in subimago females are not in agreement with the low concentrations in eggs. This weak maternal transfer could be explained by the lipid content and composition of eggs, by the mechanisms of lipid transport and by the

structure of the eggshell. In freshwater ecosystems, maternally inherited OCs in eggs have been studied mostly in fish (Svendsen et al., 2007; Volta et al., 2009) and reptiles (Rauschenberger et al. , 2004; Kelly et al., 2008) but, as far as we know, just one laboratory study with eggs of aquatic insects has been done and none in field conditions. Standley et al. (1995) found that the chlorinated compound chlordane was highly transferred to eggs in the mayfly *Centroptilum triangulifer* in laboratory conditions, contrarily to our results. These differences between mayflies could be explained by the thickness of the eggshell as a strategy related to the biology of the insect. Thus, Gaino & Rebora (2005) observed that oviparous species as *Baetis rhodani* presented thick eggshells since the egg has to survive in the environment, while ovoviviparous and parthenogenic *Cloeon dipterum* presented a very thin layer since the embryos develop inside the mother. Thus, as *C. triangulifer* is parthenogenic (Standley et al., 1995) and *E. virgo* is oviparous (Hinton, 1981), the *E. virgo* egg protection must be thicker. Moreover, *E. virgo* ovulation occurs in female nymphs about to emerge (late instar) and in the subimago, thus the eggs could be under a lower exposure time, resulting in lower OC levels. On the other hand, since the polarity of lipids determines the affinity for binding non-polar contaminants as OCs, the proportion of polar and non-polar lipids at different life cycle stages could be important to understand the observed patterns. For instance, mayfly eggs contain more than a 65 % of proteins and a 25% of lipids (Meyer et al., 1990). Both proteins and lipids are internally transported from the fat body to the oocytes via hemolymph by two main lipoproteins, vitellogenin and lipophorin (Tufail et al., 2009), and stored as protein yolk and lipid bodies (Ziegler & Antwerpen, 2006). While vitellogenin is the most abundant yolk protein precursor and transports low lipid content into the oocyte, lipophorins transport up to a 50% of lipids, mostly diacylglycerol. For instance, in fish eggs, low DDT accumulation was observed in the yolk since the precursor vitellogenin transports the most polar phospholipids with low OC affinity (Ungerer & Thomas, 1996). In the same study, DDT bounded to lipoproteins rich in non-polar triglycerides and a high DDT concentration was found in eggs. Therefore, the lipid composition bound to lipoproteins determined the affinity for binding non-polar contaminants. The egg lipid content in insects is mainly formed by triglycerides, whereas lipophorine transports diacylglycerol (Ziegler & Antwerpen, 2006) and not triacylglicerol as in the case of lipoprotein in fish. Thus, the transport of a specific type of lipids to the oocyte could explain our results for low OC bioaccumulation in eggs.

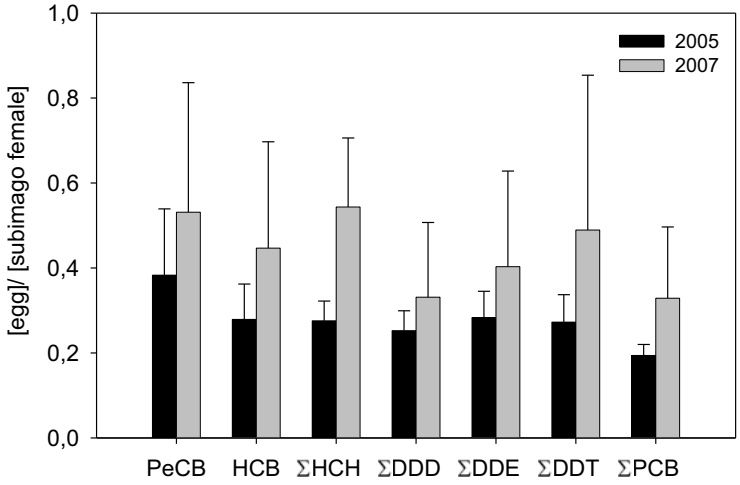


FIGURE 3. Ratio [egg]/ [subimago female] of the organochlorine compounds in 2005 and 2007.

The OC transfer to emergent *E. virgo* was evaluated using a ratio between the last instar nymphs and the male imagoes and female subimagoes in 2005. In general, the concentration change in emergent organisms was notable, with mean ratio values from 1.3 to to 6-fold higher depending on the compound (Figure 4). The highest concentration changes were observed for ∑DDE and HCB in subimago females while for males were ∑DDE, with values 6 fold higher than in the prior nymph stage. No PeCB was detected in 2005 in nymphs and imago males, and therefore no transfer ratio could be calculated. We detected that ∑HCH, ∑DDE and ∑PCB had an increase from the last nymph stage to the adults 2-fold higher in imago males than in subimago females (Figure 4). Adults contained significantly higher DDTs and DDEs than nymphs ($p<0.05$) but for DDDs, PCBs, HCHs and HCB only males were significantly higher (see Table SI2). This shows that sexual differences in OC bioaccumulation was observed for several compounds, as 4,4' DDE, PCB#101, PCB#118, PCB#138, PCB#153 and PCB#180 which presented significantly higher concentrations in imago males than in subimago females ($p<0.05$), and thereby obtaining a higher transfer in males.

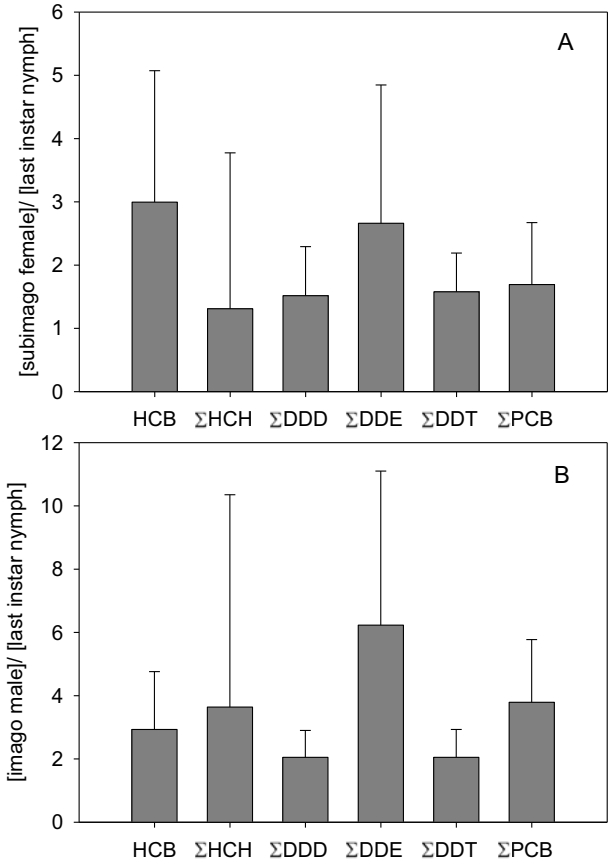


FIGURE 4. Ratio[subimago female]/ [last instar nymph] (A) and ratio[adult male/ last instar nymph] (B) of the organochlorine compounds in 2005.

Taking into account that subimagos and imagos of *E. virgo* do not feed, the high adult OC concentrations compared to nymphs might be due to a decrease in lipid content used as energy that lead to a concentration of OCs per dry weight in the adult tissues. The sexual differences can be also explained by the total amount of lipids present in males and females. Sartori et al. (1992) described that mayfly adult males used a 52% of lipids for flying while females used no significant fat. According to this, Cavaletto et al. (2003) observed a higher % of lipids in subimago males (stage prior to emergence) than in subimago females since males would need more energy for the flight. In the case of *E. virgo*, male subimago moults to imago and swarms during approximately 20 minutes until females emerge. Females emerge, mate and ovoposit as subimagos, avoiding the last moulting and the waiting flight of males, and therefore they should have less energetic requirements than males. Thus, the higher use of lipid as energy for flying can lead to a higher OC concentration in males since we captured them when they were already swarming. In addition, since subimago females analyzed in this study were

sampled plenty of eggs, the lower egg OC concentration may have diluted their total body burden.

Ecological implications of OC bioaccumulation in *E. virgo*

In general, the use of the pollution sensitive species *E. virgo* revealed the potential ecological effects of chemical contaminants on the benthic community and the use of this species as indicator is of special concern for the protection and control of the ecological status of the lower Ebro River, as suggested previously by Klok and Kraak (2008) for the Rhine River floodplain. The pollutant load detected in this keystone species evidenced the ecological risk of the lower part of the Ebro River in a context where the objectives of the Water Framework Directive 60/2000/CE (European Commission, 2000) and the European Directive 2006/11/CE have to be implemented to achieve the good ecological status of aquatic ecosystems. Thus, in order to recover the good ecological status, a restoration plan consisting in the sediment removal from the Flix reservoir should be achieved.

The variability in OC concentrations during the life cycle has some wider implications for the food web transfer in the aquatic ecosystem. In the nymph stage, the results showed that older nymphs may transfer high OCs concentration to higher tropic levels. A higher body size of aquatic invertebrates, in our case the older nymphs, increases the predation risk by insectivorous fish (Ware, 1972) and therefore the risk of OC bioaccumulation. However, since *E. virgo* nymphs burrow in the riverbed they can avoid predation better than the subimago and adult stages, but they become easily available to predators when emergences occur. Emergences of *E. virgo* in the lower Ebro can last one month (Cid et al., 2008), a relatively short period compared to the 4 months of the nymph stage. However, the strong evidence of higher bioaccumulation of OCs in adults together with an increased predation risk during emergences can lead to an important contaminant transfer to the aquatic and riparian ecosystems, affecting insectivorous vertebrates. Very high PCB levels were detected in nestling swallows fed mainly on adult mayflies during the period of mass emergences in Ontario (Smits et al., 2005), evidencing the need to study the OC patterns along the life cycle when assessing the food web transfer of these compounds. The mobilization of OC compounds from the aquatic to the terrestrial environment by emerging insects has been previously reported

(Larsson, 1984; Fairchild, 1992; Steingraeber, 1994; Corkum et al., 1997). In addition, since *E. virgo* adults can travel more than 50 km from the emergence site due to its aerial passive dispersal strategy (Ibáñez et al., 1991), they can export OCs to long distances depending on the wind speed and direction.

OC burden in eggs was low compared to the mothers or the last instar stages of nymphs, however its effect on the hatching success or on the new offspring development is not known. As far as we know, no studies on the effect of OC in eggs transferred by progenitors are available for aquatic insects. In addition, the high OC burden in late nymphs and subimago females could negatively affect fecundity, a sublethal effect reported for other pollutants as metals (Conley et al., 2009).

Since many factors can affect the OC patterns along the life cycle, as the OC bioavailability during growth, route of exposure and the ecology and physiology of the insect, further research under controlled laboratory conditions should be achieved for a better understanding of OC bioaccumulation dynamics. However, this study showed the complex patterns of bioaccumulation of OC compounds as a function of the biology and life history of insects and the uptake pathways when assessing the risk of pollutant transfer to higher trophic levels in aquatic ecosystems.

Acknowledgements

This work was supported by the Government of Spain (Ministerio de Educación y Ciencia, research project CGL2006-01487, Plan Nacional I+D+I; Ministerio de Medio Ambiente y Medio Rural y Marino; Consejo Superior de Investigaciones Científicas), the Government of Catalonia (Departament d'Innovació, Universitats i Empresa; Departament de Medi Ambient i Habitatge; Agència Catalana de l'Aigua) and by the European Social Fund. We are very grateful to D. Mateu, R. Andreu, L. Jornet, M. Cid, B. Lefler, R. Valmaña for their technical assistance.

Supporting Information Available

Table SI1 contains data of OCs concentration for each compound and statistical analysis related to nymph growth. Table SI2 contains the statistical results of

polynomial orthogonal contrasts on OC concentrations according to nymph size in 2005. Table SI3 contains data of OCs concentration for each compound and statistical analysis related to the different life cycle stages.

Literature Cited

(1) Barata, C.; Alanon, P.; Gutiérrez-Alonso, S.; Riva, M. C.; Fernández, C.; Tarazona, J. V., A *Daphnia magna* feeding bioassay as a cost effective and ecological relevant sublethal toxicity test for Environmental Risk Assessment of toxic effluents. *Sci Total Environ* **2008,** *405*, (1-3), 78-86.

(2) Bartrons, M.; Grimalt, J. O.; Catalán, J., Concentration changes of organochlorine compounds and polybromodiphenyl ethers during metamorphosis of aquatic insects. *Environ Sci Technol* **2007,** *41*, (17), 6137-6141.

(3) Bonada, N.; Vives, S.; Rieradevall, M.; Prat, N., Relationship between pollution and fluctuating asymmetry in the pollution-tolerant caddisfly *Hydropsyche exocellata* (Trichoptera, Insecta). *Archiv Fur Hydrobiologie* **2005,** *162*, (2), 167-185.

(4) Bosch, C.; Olivares, A.; Faria, M.; Navas, J.M.; del Olmo, I.; Grimalt, J.O.; Piña, B.; Barata, C. Identification of water soluble and particle bound compounds causing sublethal toxic effects. A field study on sediments affected by a chlor-alkali industry", *Aquatic Toxicol* **2009**, *94*, (1), 16-27.

(5) Cain, D. J.; Luoma, S. N.; Carter, J. L.; Fend, S. V., Aquatic Insects as Bioindicators of Trace-Element Contamination in Cobble-Bottom Rivers and Streams. *Can J Fish Aquat Sci* **1992,** *49*, (10), 2141-2154.

(6) Campbell, L. M.; Schindler, D. W.; Muir, D. C. G.; Donald, D. B.; Kidd, K. A., Organochlorine transfer in the food web of subalpine Bow Lake, Banff National Park. *Can J Fish Aquat Sci* **2000,** *57*, (6), 1258-1269.

(7) Catalán, J.; Ventura, M.; Vives, I.; Grimalt, J. O., The roles of food and water in the bioaccumulation of organochlorine compounds in high mountain lake fish. *Environ Sci Technol* **2004,** *38*, (16), 4269-4275.

(8) Cavaletto, J. F.; Nalepa, T. F.; Fanslow, D. L.; Schloesser, D. W., Temporal variation of energy reserves in mayfly nymphs (*Hexagenia* spp.) from Lake St Clair and western Lake Erie. *Freshw Biol* **2003,** *48*, (10), 1726-1738.

(9) Cid, N.; Ibáñez, C.; Prat, N., Life history and production of the burrowing mayfly *Ephoron virgo* (Olivier, 1791) (Ephemeroptera: Polymitarcyidae) in the lower Ebro River: A comparison after 18 years. *Aquat Insects* **2008,** *30*, (3), 163-178.

(10) Cid, N.; Ibáñez, C.; Prat, N., Patterns of metal bioaccumulation in two filter-feeding macroinvertebrates: exposure distribution, inter-species differences and variability across developmental stages. *Sci Total Environ* **2010,** *408*, (14), 2795–2806.

(11) Conley, J. M.; Funk, D. H.; Buchwalter, D. B., Selenium Bioaccumulation and Maternal Transfer in the Mayfly *Centroptilum triangulifer* in a Life-Cycle, Periphyton-Biofilm Trophic Assay. *Environ Sci Technol* **2009,** *43*, (20), 7952-7957.

(12) Corkum, L. D.; Ciborowski, J. J. H.; Lazar, R., The distribution and contaminant burdens of adults of the burrowing mayfly, *Hexagenia*, in Lake Erie. *J Gt Lakes Res* **1997,** *23*, (4), 383-390.
(13) Day, R. W.; Quinn, G. P., Comparisons of treatments after an analysis of variance in ecology. *Ecol Monogr* **1989,** *59*, (4), 433-463.
(14) De Haas, E. M.; Kraak, M. H. S., Species-specific responses of two benthic invertebrates explain their distribution along environmental gradients in freshwater habitats. *Sci Total Environ* **2008,** *406*, (3), 430-435.
(15) De Haas, E. M.; Reuvers, B.; Moermond, C. T. A.; Koelmans, A. A.; Kraak, M. H. S., Responses of benthic invertebrates to combined toxicant and food input in floodplain lake sediments. *Environ Toxicol Chem* **2002,** *21*, (10), 2165-2171.
(16) De Lange, H. J.; De Haas, E. M.; Maas, H.; Peeters, E., Contaminated sediments and bioassay responses of three macroinvertebrates, the midge larva *Chironomus riparius*, the water louse *Asellus aquaticus* and the mayfly nymph *Ephoron virgo*. *Chemosphere* **2005,** *61*, (11), 1700-1709.
(17) Directive 2006/11/EC of the European Parliament and of the Council of 15 February 2006 on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community. Official Journal 4 March 2006 L 64/52. European Commission, Brussels.
(18) Drouillard, K. G.; Ciborowski, J. J. H.; Lazar, R.; Haffner, G. D., Estimation of the uptake of organochlorines by the mayfly *Hexagenia limbata* (Ephemeroptera: Ephemeridae). *J Gt Lakes Res* **1996,** *22*, (1), 26-35.
(19) Edsall, T. A.; Manny, B. A.; Schloesser, D. W.; Nichols, S. J.; Frank, A. M., Production of *Hexagenia limbata* nymphs in contaminated sediments in the Upper Great-Lakes connecting channels. *Hydrobiologia* **1991,** *219*, 353-361.
(20) Eljarrat, E.; Martínez, M. A.; Sanz, P.; Concejero, M. A.; Piña, B.; Quirós, L.; Raldúa, D.; Barceló, D., Distribution and biological impact of dioxin-like compounds in risk zones along the Ebro River basin (Spain). *Chemosphere* **2008,** *71*, (6), 1156-1161.
(21) European Commission (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy. Official Journal 22 December 2000 L 327/1. European Commission, Brussels.
(22) Fairchild, W. L.; Muir, D. C. G.; Currie, R. S.; Yarechewski, A. L., Emerging insects as biotic pathway for movement of 2,3,7,8- Tetrahchlorodibenzofuran from lake sediments. *Environ Toxicol Chem* **1992,** *11*, (6), 867-872.
(23) Fernández, M. A.; Alonso, C.: González, Mª. J.; Hernández, L. M. Occurence of organochlorine insecticides, PCBs and PCB congeners in waters and sediments of the Ebro River (Spain). *Chemosphere* **1999**, 38, (1), 33-43.
(24) Gaino, E.; Rebora, M., Egg envelopes of *Baetis rhodani* and *Cloeon dipterum* (Ephemeroptera, Baetidae): a comparative analysis between an oviparous and an ovoviviparous species. *Acta Zoologica* **2005,** *86*, (1), 63-69.
(25) Gómez-Gutiérrez, A. I.; Jover, E.; Bodineau, L.; Albaiges, J.; Bayona, J. M., Organic contaminant loads into the Western Mediterranean Sea: Estimate of Ebro River inputs. *Chemosphere* **2006,** *65*, (2), 224-236.
(26) Grimalt, J.O. 2006. http://mediambient.gencat.cat/cat/ciutadans/informacio_ambiental/Flix/inici.jsp
(27) Hahn, T.; Schenk, K.; Schulz, R., Environmental chemicals with known endocrine potential affect yolk protein content in the aquatic insect *Chironomus riparius*. *Environ Pollut* **2002,** *120*, (3), 525-528.

(28) Hinton, H. E., *Biology of insect eggs* Pergamon Press: Oxford, New York, Toronto, Sydney, Paris, Frankfurt, **1981**; Vol. 2, p 1125.
(29) Holt, M. S. Sources of chemical contaminants and routes into the freshwater environment. *Food Chem Toxicol* **2000**, *38*, S21-S27.
(30) Ibáñez, C.; Escosa, R.; Muñoz, I.; Prat, N., Life cycle and production of Ephoron *virgo* (Ephemeroptera: Polymitarcyidae) in the lower River Ebro (NE Spain). In *Overview and strategies of Ephemeroptera and Plecoptera*, Alba-Tercedor, J., Sánchez-Ortega, A., Ed. The Sandhill Crane Press: Gainesville, Florida, U.S.A., **1991**; pp 483-492.
(31) Karouna-Renier, N. K.; Sparling, D. W., Relationships between ambient geochemistry, watershed land-use and trace metal concentrations in aquatic invertebrates living in stormwater treatment ponds. *Environ Pollut* **2001,** *112*, (2), 183-192.
(32) Kelly, S. M.; Eisenreich, K. M.; Baker, J. E.; Rowe, C. L., Accumulation and maternal transfer of polychlorinated biphenils in snapping turtles of the upper Hudson River, New York, USA. *Environ Toxicol Chem* **2008,** *27*, (12), 2565-2574.
(33) Kidd, K. A.; Hesslein, R. H.; Ross, B. J.; Koczanski, K. Stephens, G. R.; Muir, D. C. G. Bioaccumulation of organochlorines through a remote freshwater food web in the Canadian Artic. *Environ. Pollut.* **1998**, *102*, (1), 91-103.
(34) Klok, C.; Kraak, M. H. S., Living in highly dynamic polluted river floodplains, do contaminants contribute to population and community effects? *Sci Total Environ* **2008,** *406*, (3), 455-461.
(35) Kureck, A.; Fontes, R. J., The life cycle and emergence of *Ephoron virgo*, a large potamal mayfly that has returned to the River Rhine. *Arch Hydrobiol Suppl* **1996,** *113*, (1-4), 319-323.
(36) Lacorte, S.; Raldúa, D.; Martínez, E.; Navarro, A.; Díez, S.; Bayona, J. M.; Barceló, D., Pilot survey of a broad range of priority pollutants in sediment and fish from the Ebro river basin (NE Spain). *Environ Pollut* **2006,** *140*, (3), 471-482.
(37) Larsson, P., transport of PCBs from aquatic to terrestrial environments by emerging chironomids. *Environmental Pollution Series a-Ecological and Biological* **1984,** *34*, (3), 283-289.
(38) Lavado, R.; Urena, R.; Martin-Skilton, R.; Torreblanca, A.; del Ramo, J.; Raldúa, D.; Porte, C., The combined use of chemical and biochemical markers to assess water quality along the Ebro River. *Environ Pollut* **2006,** *139*, (2), 330-339.
(39) Malmqvist, B.; Rundle, S. Threats to the running water ecosystems of the world. *Environ Conserv* **2002**, *29* (2), 134-153.
(40) Medchem (1996). Database and calculation method for Kow values.
(41) Meyer, E., Levels of major body compounds in nymphs of the stream mayfly *Epeorus sylvicola* (Pict) (Ephemeroptera, Heptageniidae). *Archiv Fur Hydrobiologie* **1990,** *117*, (4), 497-510.
(42) Morrissey, C. A.; Bendell-Young, L. I.; Elliott, J. E., Identifying sources and biomagnification of persistent organic contaminants in biota from mountain streams of southwestern British Columbia, Canada. *Environ Sci Technol* **2005,** *39*, (20), 8090-8098.
(43) Paumen, M. L.; Borgman, E.; Kraak, M. H. S.; van Gestel, C. A. M.; Admiraal, W., Life cycle responses of the midge *Chironomus riparius* to polycyclic aromatic compound exposure. *Environ Pollut* **2008,** *152*, (1), 225-232.

(44) Rauschenberger, R. H.; Sepulveda, M. S.; Wiebe, J. J.; Szabo, N. J.; Gross, T. S., Predicting maternal body burdens of organochlorine pesticides from eggs and evidence of maternal transfer in *Alligator mississippiensis*. *Environ Toxicol Chem* **2004,** *23*, (12), 2906-2915.
(45) Sartori, M.; Keller, L.; Thomas, A. G. B.; Passera, L., Flight energetics in relation to sexual differences in the mating-behavior of a mayfly, *Siphlonurus-aestivalis*. *Oecologia* **1992,** *92*, (2), 172-176.
(46) Skinner, K. M.; Bennett, J. D., Altered gill morphology in benthic macroinvertebrates from mercury enriched streams in the Neversink Reservoir Watershed, New York. *Ecotoxicology* **2007,** *16*, (3), 311-316.
(47) Smits, J. E. G.; Bortolotti, G. R.; Sebastian, M.; Ciborowski, J. J. H., Spatial, temporal, and dietary determinants of organic contaminants in nestling tree swallows in Point Pelee National Park, Ontario, Canada. *Environ Toxicol Chem* **2005,** *24*, (12), 3159-3165.
(48) Smock, L. A., Relationships between Metal Concentrations and Organism Size in Aquatic Insects. *Freshw Biol* **1983,** *13*, (4), 313-321.
(49) Sokal, R. R.; Rohlf, F. J., *Biometry: The Principles and Practice of Statistics in Biological Research.* New York, 1995.
(50) Standley, L. J.; Sweeney, B. W., Organochlorine pesticides in stream mayflies and terrestrial vegetation of undisturbed tropical catchments exposed to long-range atmospheric transport. *J North Am Benthol Soc* **1995,** *14*, (1), 38-49.
(51) Standley, L. J.; Sweeney, B. W.; Funk, D. H., Maternal transfer of chlordane and its metabolites to eggs of a stream mayfly *Centroptilum triangulifer*. *Environ Sci Technol* **1994,** *28*, (12), 2105-2111.
(52) Steingraeber, M. T.; Schwartz, T. R.; Wiener, J. G.; Lebo, J. A., Polychlorinated biphenyl congeners in emergent mayflies from the upper Mississippi River. *Environ Sci Technol* **1994,** *28*, (4), 707-714.
(53) Steingraeber, M. T.; Wiener, J. G., Bioassessment of contaminant transport and distribution in aquatic ecosystems by chemical analysis of burrowing mayflies (*Hexagenia*). *Regulated Rivers-Research & Management* **1995,** *11*, (2), 201-209.
(54) Svendsen, T. C.; Vorkamp, K.; Frederiksen, M.; Ronsholdt, B.; Frier, J. O., Body burdens of persistent halogenated compounds during different development stages of anadromous brown trout (*Salmo trutta*). *Environ Sci Technol* **2007,** *41*, (17), 5980-5985.
(55) Terrado, M.; Barceló, D.; Tauler, R., Identification and distribution of contamination sources in the Ebro basin by chemometrics modelling coupled to geographical information systems. *Talanta* **2006,** *70*, (4), 691-704.
(56) Torralba-Burial, A.; Ocharan, F. J., Distribución de *Ephoron virgo* (Ephemeroptera: Polymitarcyidae) en Aragón (NE España). *Boletin de la Sociedad Entomológica de Aragón* **2004,** *35*, 203-206.
(57) Tufail, M.; Takeda, M., Insect vitellogenin/lipophorin receptors: Molecular structures, role in oogenesis, and regulatory mechanisms. *J Insect Physiol* **2009,** *55*, (2), 87-103.
(58) Ungerer, J. R.; Thomas, P., Role of very low density lipoproteins in the accumulation of o,p'-DDT in fish ovaries during gonadal recrudescence. *Aquat Toxicol* **1996,** *35*, (3-4), 183-195.
(59) Van der Geest, H. G.; Greve, G. D.; Kroon, A.; Kuijl, S.; Kraak, M. H. S.; Admiraal, W., Sensitivity of characteristic riverine insects, the caddisfly *Cyrnus trimaculatus* and the mayfly *Ephoron virgo*, to copper and diazinon. *Environ Pollut* **2000,** *109*, (2), 177-182.

(60) Vives, I.; Grimalt, J. O., Method for integrated analysis of polycyclic aromatic hydrocarbons and organochlorine compounds in fish liver. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* **2002,** *768*, (2), 247-254.
(61) Vives, I.; Grimalt, J. O.; Ventura, M.; Catalán, J., Distribution of polycyclic aromatic hydrocarbons in the food web of a high mountain lake, Pyrenees, Catalonia, Spain. *Environ Toxicol Chem* **2005a,** *24*, (6), 1344-1352.
(62) Vives, I.; Grimalt, J. O.; Ventura, M.; Catalán, J.; Rosseland, B. O. (2005). Age dependence of the accumulation of organochlorine pollutants in brown trout (*Salmo trutta* , Pyrenees). *Environ Pollut* **2005b**, *133,* (2), 343-350.
(63) Volta, P.; Tremolada, P.; Neri, M. C.; Giussani, G.; Galassi, S., Age-Dependent bioaccumulation of organochlorine compounds in fish and their selective biotransformation in top predators from Lake Maggiore (Italy). *Water Air Soil Pollut* **2009,** *197*, (1-4), 193-209.
(64) Ware, D. M., Predation by rainbow trout (*Salmo gairdneri*): influence of hunger, prey density and prey size. *J Fish Res Board Can* **1972,** *29*, (8), 1193-&.
(65) Ziegler, R.; Van Antwerpen, R., Lipid uptake by insect oocytes. *Insect Biochem Mol Biol* **2006,** *36*, (4), 264-272.

Supporting Information

Table SI1. Data of OCs concentration for each compound and statistical analysis during nymph growth.

	HCB	γ-HCH	PCB28	PCB52	PCB101	PCB118	PCB138	PCB153	PCB180	∑PCB	2,4'-DDE	4,4'-DDE	∑DDE	2,4'-DDD	4,4'-DDD	∑DDD	2,4'-DDT	4,4'-DDT	∑DDT
29-May-05	18 ± 1ab	nd	nd	nd	3 ± 5a	17 ± 6a	42 ± 3ab	38 ± 3ab	51 ± 1ab	134 ± 4ab	nd	141 ± 12a	141 ± 12a	27 ± 4ab	30 ± 7ab	74±14a	34 ± 8a	64 ± 21a	148 ± 41a
12-Jun-05	21 ± 3a	nd	nd	nd	nd	nd	32 ± 3a	26 ± 3a	29.3 ± 0.6a	91 ± 5a	nd	80 ± 2a	80 ± 2a	13 ± 2a	27 ± 6ab	32±6a	24 ± 5a	40 ± 4a	62 ± 7a
04-Jul-05	52 ± 4ab	8 ± 7a	10 ± 4a	nd	13 ± 3a	10 ± 1a	47 ± 1ab	47 ± 0ab	42 ± 2ab	163 ± 8ab	3.2 ± 0.0	282 ± 53ab	286 ± 58ab	17 ± 4ab	24 ± 3a	42±7a	25 ± 6a	80 ± 6a	108 ± 0a
04-Aug-05	83 ± 13b	28 ± 16a	21 ± 6a	1 ± 1	17 ± 8a	24 ± 1a	55 ± 1b	80 ± 2b	45 ± 3b	241 ± 15b	nd	482 ± 62b	448 ± 28b	25 ± 2b	47 ± 4b	72±2a	43 ± 3a	99 ± 10a	142 ± 6a

Numbers followed by different letter in the column present a significant difference ($p < 0.05$). nd: not detected.

Table SI2. Statistical results of polynomial orthogonal contrasts on OC concentration in relationship with nymph size for 2005.

data Polynomial Contrasta			Dependent Variable																						
			PeCB	a_HCH	HCB	b_HCH	g_HCH	d_HCH	PCB28	PCB52	24_DDE	PCB_101	44_DDE	24_DDD	PCB118	44_DDD	24_DDT	PCB153	44_DDT	PCB138	PCB180	HCHs	DDDs	DDEs	DDTs	PCBs
Linear	Contrast Estimate		.000	.000	.415	.000	1.124	.000	1.002	.224	.144	.686	.482	.060	.739	.036	.013	.227	.068	.072	.039	1.087	.043	.482	.051	.179
	Hypothesized Value		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Difference (Estimate -		.000	.000	.415	.000	1.124	.000	1.002	.224	.144	.686	.482	.060	.739	.036	.013	.227	.068	.072	.039	1.087	.043	.482	.051	.179
	Std. Error		.000	.000	.073	.000	.431	.000	.275	.167	.162	.338	.071	.053	.264	.073	.065	.054	.093	.045	.043	.417	.062	.071	.081	.049
	Sig.		.	.	.000	.	.031	.	.007	.217	.397	.077	.000	.292	.023	.641	.850	.003	.486	.146	.389	.031	.510	.000	.546	.007
	95% Confidence Interval for Difference	Lower Bound	.000	.000	.246	.000	.129	.000	.367	-.161	-.228	-.093	.318	-.063	.130	-.134	-.137	.103	-.147	-.031	-.060	.126	-.100	.318	-.136	.066
		Upper Bound	.000	.000	.584	.000	2.118	.000	1.637	.609	.517	1.466	.646	.183	1.347	.205	.162	.351	.283	.175	.139	2.049	.185	.647	.239	.292

Table SI3. Data of OCs concentration for each compound and statistical analysis across the different life cycle stages.

2005	PeCB	HCB	γ-HCH	HCHs	PCB28	PCB52	PCB101	PCB118	PCB138	PCB153	PCB180	PCBs	2,4'-DDE	4,4'-DDE	DDE	2,4'-DDD	4,4'-DDD	DDD	2,4'-DDT	4,4'-DDT	DDT
Eggs	1.6 ± 0.6a	32 ± 8a	3.1 ± 0.4a	3.1 ± 0.4a	8 ± 2a	nd a	6 ± 2a	10 ± 3a	10 ± 3a	17 ± 5a	8 ± 2a	53 ± 14a	1.0 ± 0.4a	159 ± 43a	160 ± 43a	7 ± 2a	14 ± 4a	21 ± 6a	11 ± 3a	35 ± 10a	47 ± 13a
Nymphs	nd b	42 ± 25a	7 ± 12a	7 ± 12a	6 ± 9a	0.2 ± 0.8a	6 ± 7a	47 ± 12ab	47 ± 12b	52 ± 22b	42 ± 11b	164 ± 62b	0.5 ± 1a	238 ± 164a	239 ± 164a	19 ± 6b	33 ± 14b	52 ± 19b	34 ± 12b	73 ± 26a	107 ± 37b
Subimago female	5 ± 2c	124 ± 43b	8 ± 5ab	9 ± 5ab	34 ± 10b	1 ± 3a	23 ± 10b	61 ± 29bc	61 ± 29b	89 ± 46bc	48 ± 22b	278 ± 122bc	14 ± 15b	621 ± 273b	635 ± 287b	30 ± 13b	49 ± 16bc	79 ± 28bc	45 ± 16bc	123 ± 18b	169 ± 30c
Adult male	nd b	122 ± 20b	19 ± 3b	19 ± 3b	36 ± 3b	17 ± 9a	80 ± 13b	62 ± 3c	167 ± 20b	261 ± 16c	121 ± 7b	748 ± 84c	16 ± 0.2b	1746 ± 343b	1773 ± 357b	62 ± 9b	57 ± 1c	118 ± 8c	85 ± 21c	122 ± 0.2b	187 ± 9c

2007	PeCB	HCB	γ-HCH	HCHs	PCB28	PCB52	PCB101	PCB118	PCB138	PCB153	PCB180	PCBs	2,4'-DDE	4,4'-DDE	DDEs	2,4'-DDD	4,4'-DDD	DDDs	2,4'-DDT	4,4'-DDT	DDTs
Eggs	1.3 ± 0.6ab	30 ± 18a	0.6 ± 0.4a	2 ± 1ab	12 ± 7a	7 ± 4ab	11 ± 6a	7 ± 4a	21 ± 12a	33 ± 18a	18 ± 10a	107 ± 60a	3 ± 2a	272 ± 147a	275 ± 149a	11 ± 6a	17 ± 9a	28 ± 15a	29 ± 17ab	105 ± 64a	134 ± 81a
Nymphs	0.4 ± 0.5a	20 ± 1a	0.7 ± 0.9ab	1 ± 1a	17 ± 3a	4 ± 5a	25 ± 6b	18 ± 13a	76 ± 7b	90 ± 20b	69 ± 10b	299 ± 48a	5 ± 3ab	195 ± 49a	199 ± 51a	28 ± 6b	29 ± 12ab	57 ± 18b	49 ± 16a	135 ± 71a	184 ± 85a
Subimago female	3 ± 2b	68 ± 47a	1.4 ± 0.8ab	3 ± 1a	33 ± 21ab	21 ± 12ab	42 ± 25bc	24 ± 12ab	98 ± 60abc	134 ± 80abc	84 ± 50abc	366 ± 224ab	8 ± 5ab	955 ± 582ab	790 ± 490ab	46 ± 27ab	62 ± 39ab	123 ± 72ab	84 ± 49ab	347 ± 202a	431 ± 674a
Adult male	3 ± 2b	86 ± 52a	3 ± 2b	5 ± 3b	59 ± 34b	48 ± 25b	94 ± 50c	66 ± 36b	194 ± 102c	301 ± 156c	162 ± 83c	925 ± 481b	16 ± 8b	1779 ± 896b	1794 ± 904b	61 ± 33b	35 ± 18b	96 ± 51b	103 ± 54b	178 ± 90a	281 ± 144a

Numbers followed by different letter in the column present a significant difference ($p < 0.05$).

General discussion

The present discussion aims to give a general perspective of the main issues discussed along the previous chapters and justify the conclusions. Since the main human impacts in the Ebro River were already described in the introduction, this section was focused on the two main impacts considered: the assessment and effects of hydrological alterations and the the relevance of heavy metal and of organochlorine pollution (OC), by using benthic macroinvertebrates. In general, the present thesis demonstrated that the freshwater ecosystem of the lower Ebro River is hydrologically altered and suffers an important anthropogenic stress, experiencing an elevated bioaccumulation of the existing pollutants in selected key species, which resulted in changes in the structure and function of the community.

In highly regulated rivers as the Ebro, the hydrodynamic conditions are altered affecting the composition and diversity of aquatic communities downstream in many direct and indirect ways, including longitudinal habitat fragmentation, habitat homogenization, impairment of water quality and the presence of invasive species. In this context, the use of macroinvertebrates as indicators of hydrological alterations has a long tradition of study and use for management, including works to implement environmental flows (Gore, 1978; Gore, 2001; Suren & Jowett, 2006; James & Suren, 2009; Dunbar et al., 2010).

In **Chapter 1** and **2**, the response of the macroinvertebrate community to different hydrological and hydraulic situations was assessed, either considering the taxonomy and functional trait-based approaches. In **Chapter 1**, the temporal variation according to different hydrological conditions was highly reflected in the functional trait composition

and metrics. A previous situation of relatively high flow in spring determined a community with a lower functional diversity than in autumn, since the previous long period of stable and relatively low flows before October provided a higher functional diversity. However, for clarity in the interpretation of the functional trait response to flow variation, studies using direct water physical measurements such as water veolocity or shear stress are required (Statzner & Bêche, 2010). This idea was approached in **Chapter 2,** showing that most of the trait categories considered in the study responded significantly (positively or negatively) to water velocity, Reynolds and Froude number. Several studies have investigated the response of the trait composition to hydraulic conditions (Lamoroux et al., 2004; De Crespin et al., 2002; Snook & Milner, 2002; Mérigoux & Dolédec, 2004; Tomanova & Usseglio-Polatera, 2007; Horrigan & Baird, 2008), demonstrating that traits are a primary filter which determine which species can survive and reproduce under certain environmental conditions (Habitat templet theory; Southwood, 1988; Poff &Ward, 1990; Townsend & Hildrew, 1994), and the importance of the physical habitat structuring the assemblages of aquatic invertebrates in running waters.

As considered for the biological trait approach, a good understanding of the ecological niche of species based on direct hydraulic measurements would provide more specific responses to flow variations. Thus, the hydraulic requirements of species and the response of their biological traits could provide a basis for their application in guiding river flow management (Gore et al., 2001), considering that functional responses at microhabitat level can predict reach-scale responses (Lamoroux et al., 2004) and that the species presenting high habitat hydraulic marginality would be those more sensitive to hydrological alterations (Dolédec et al., 2007) . Moreover, the higher the taxonomic resolution, the more precise will be our understanding of their response according to the hydraulic conditions since different responses were obtained for same genus (e.g., *Cricotopus (C.) trifascia* and *C. (C.) bicinctus*) (**Chapter 2**).

Chapter 1 and **2** are one of the few studies comparing simultaneously structural and functional changes in the macroinvertebrate community in large rivers at different spatial scales, although this effort has been previously done in other studies considering large datasets from large spatial scales in Europe and North America (Bady et al., 2005; Bonada et al., 2007; Bêche &Statzner, 2009; Péru & Dolédec, 2010).

In the context of global change, an increase of temperatures and a reduction of precipitations have been predicted in Mediterranean climates (IPCC, 2007), and the impacts on the water resources of the Ebro basin climate have been already studied (CHE, 2005). This situation together with the present and ongoing projects of water abstractions in the Ebro due to land use changes (agriculture) can lead to water scarcity issues and subsequent management problems when proposals concerning environmental flows are being considered in the Ebro (Sánchez & Ibáñez, 2008) and the Water Framework directive has to be implemented (European Commission, 2000). For the moment, changes in the phenology of terrestrial insects, birds and plants (Gordo & Sanz, 2005) and aquatic insects (**Chapter 3** of this thesis) have been detected.

The alteration of the flow regime affects both fish and macroinvertebrate community composition and enhances the introduction and establishment of alien species (Poff et al., 2010). Thus, as showed in **Chapters 1** and **2**, populations of non-native species such as *Corbicula fluminea* were dominant which could be explained by their high reproductive capacity and their low hydraulic requirements, since occupy awide range of habitat conditions. However, despite the high abundances of this filter feeder in the lower Ebro, and the high presence of zebra mussel (*D. polymorpha*) in the reservoirs, the results from secondary production after 18 years in populations of the native filter-feeder *E. virgo* showed even higher values than in 1987 (**Chapter 3**). This could probably be due to the dramatic decline of native freshwater mussel populations such as *Margaritifera auricularia* or *Unio elungatulus* C. Pfeiffer (Ramos, 1998; Araujo & Ramos, 2000), not detected in any of the samples collected during the present study, since the filtration action of *Corbicula* could have replaced the previous competition with naiads.

In **Chapters 4** and **5** the bioaccumulation of heavy metals and organochlorine compounds in benthic macroinvertebrate fauna downstream of the sediment dump located at the Flix reservoir were investigated at individual/ population level. The study organisms were a tolerant (*H. exocellata*) and a sensitive benthic organism (*E. virgo*) which presented different levels of bioaccumulation when considering heavy metals. Since both species present similar biological traits regarding respiration and feeding habits, both are filter feeders and breathe by gills (Tachet et al., 2000), we considered that they would be under similar levels of exposure to contaminants, either via ingestion of suspended particles or via respiration or direct contact of dissolved pollutants.

Despite multiple stressors may act simultaneously in the lower Ebro River, the high bioaccumulation levels detected in *H. exocellata* (**Chapter 4**) and the absence or low densities of species considered as pollutant sensitive (*E. virgo*) in the first km below the sediment dump (**Chapter 1**), indicated that the changes at population level (relative abundances) were mainly attributed to chemical pollution released from the pollution source. Due to interespecific different ecophysiological traits which have a phylogenethic origin (Buchwalter et al., 2008), species with similar biological trait profile but genetically distant might differ in their ability to maintain stable populations in metal or OC polluted freshwaters. The higher metal bioaccumulation found in *E. virgo* compared to *H. exocellata* in **Chapter 4** evidenced these different ecophysiological traits. *H. exocellata* can easily detoxify and eliminate pollutants (Cain et al., 2004; Buchwalter et al., 2008) and populations did not appear to be affected by the pollution load, whereas *E. virgo* populations were adversely affected and started to be moderately abundant 21 km downstream of Flix and only highly abundant in the lowermost sampled location from the pollution source (**Chapter 3**). Accordingly, the macrobenthic structure changed as river kilometer was increasing from the pollution impact (Figure 9). Changes at community level have been widely reported in pollutant enriched freshwaters (Liess et al., 1996; Hutchens et al., 1998; Clements et al., 2000; De Lange et al., 2004; Cain et al., 2004), giving special attention to the Ephemeroptera since they usually are one of the first populations showing significant declines in abundance and richness at sites with high levels of toxic pollutants (Edsall et al., 1991; Clements et al., 2000; Maret et al., 2003). Moreover, indirect effects on sensitive populations can increase predation rate under high levels of metal or pesticide pollution (Clements, 1999; Schultz & Dabrowski, 2001) since sublethal effects can lead to behavioral changes of animals (e.g., reduced locomotion). This could be possible in the lower Ebro River, since we observed that nymphs living upstream of the sediment dump presented a higher mobility than those living downstream. Taking all this into account, *E. virgo* was a good bioindicator of pollution since it is sensitive to sediment-bound toxicants (De Haas et al., 2002) and therefore is considered a species of special concern for the protection of fluvial ecosystems in its area of distribution (Klok et al., 2008), which includes the Ebro River and most of the European large rivers. Beyond the spatial patterns of pollution, temporal changes in the fluvial ecosystem also can determine the exposure to pollutants by aquatic fauna (Chapman et al., 2003). For instance, in **Chapter 1** the taxonomy-based metrics (including richness, diversity indices and the

biotic index IBMWP) presented higher values with increasing distance from the sediment dump only in spring. This higher response in spring was due to those sensitive Ephemeroptera species such as *E. virgo* or *Choroterpes pictetii*, which are only present as nymphs in spring-summer due to their univoltine life cycle. Therefore, the life cycle patterns of key indicator species have to be considered to assess spatial and temporal patterns of pollution. Furhermore, the lower Ebro, although being a highly regulated river, a higher discharge in wet years following spring snowmelt in the beginning of spring could lead to a higher pollutant exposure due to the sediment remobilization from the Flix reservoir and from deposition areas downstream after a higher discharge.

On the other hand, functional metrics based on biological traits (trait richess, functional diversity) only detected slight changes in the community along the pollution gradient (Figure 9). This showed the difficulty to establish a mechanistic understanding of community responses by using functional metrics based only on biological traits (e.g., maximal size, feeding habits), since they do not integrate the different ecophysiological species traits which are crucial in metal polluted environments. However, the use of *a priori* predictions of selected trait modalities based on expected responses to specific types of impact could improve the cause-effect interpretation of this approach (Dolédec & Statzner, 2008). In the lower Ebro, since the main pollutants were likely incorporated via ingestion of particulate matter (**Chapter 4**), lower proportions of filter –feeders were expected in the areas closer to the pollution source when considering the whole macroinvertebrate community as an indicator. This agreed with the results from **Chapter 1**, when the trait-based approach was used to detect longitudinal functional changes from the main dams and the metal and organochlorine pollution source. To our knowledge, only two recent papers have related biological traits of aquatic invertebrates with the impact of toxic substances in rivers (Dolédec & Statzner, 2008; Archaimbault et al., 2010). Thus, it can still be difficult to interpret the response of biological traits to this specific stress. Moreover, in most of the running waters impacted by toxic pollutants other type of stressors may act simultaneously which make the scenario even more complicated.

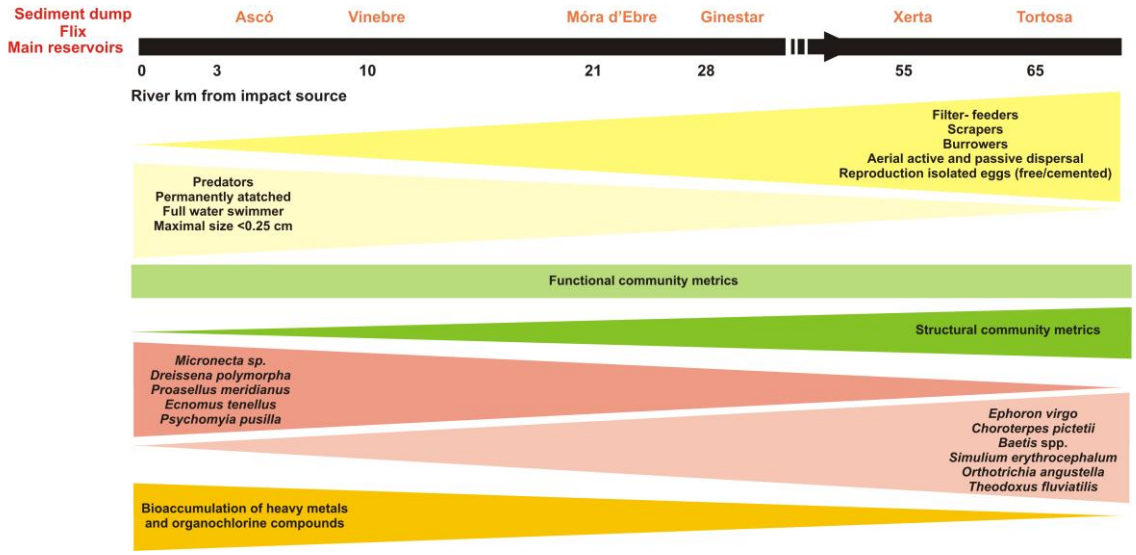


Figure 9. General longitudinal patterns observed in the macroinvertebrate community of the lower Ebro River from the sediment dump at Flix to Tortosa.

In pollutant-enriched aquatic environments, the biology and life history of aquatic insects should be considered when assessing the risk of pollutant transfer to higher trophic levels (Corkum et al., 1997; Smits et al., 2005; Bartrons et al., 2007). For this reason, in **Chapters 4** and **5** the transfer of metals and OCs was assessed across the life cycle considering *E. virgo* as a model. As studied in **Chapter 3**, the univoltine and synchronized life cycle of this species propitiated a good chance to study the transfer of pollutants along life cycle under field conditions, and therefore reflecting what happened in the natural habitat. The nymphs of E. *virgo* hatch in April, and have a fast development until emergence and reproduction occur in late July, when eggs are deposited in the river and spend the autumn and winter in a diapauses attached to river sediment (Kureck & Fontes, 1990). Due to the high secondary production of this species in the lower Ebro (annual nymph production of 950 mg·m^2·year dry weight) and to its abundant adult mass emergences, *E. virgo* is an abundant prey for insectivorous fish and birds and therefore is of special ecological relevance. Since the metal and OC fate in aquatic insects varies with growth and life cycle stages (Smock, 1983; Cain et al., 1992; Standley et al., 1994; Bartrons et al., 2007) and different bioaccumulation patterns have been described for different compounds (Smock, 1983), **Chapter 4** and **5** illustrated how the most persistent pollutants as Hg, Cd, PCBs or DDEs were highly transferred to emergent adults and to the eggs, although the latter presented much lower concentrations and in the case of Cd the transfer to eggs was minimum (Figure 10).

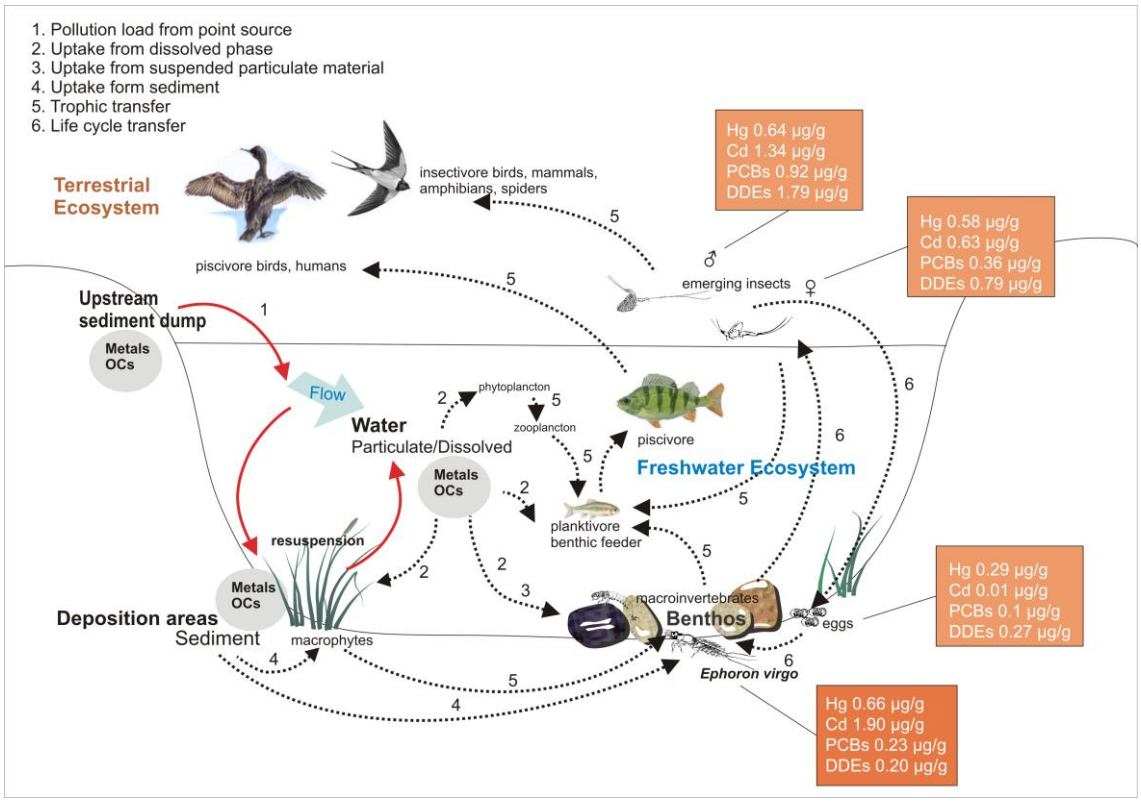


Figure 10. Pollutant transfer into the aquatic foodweb, considering the Ebro River as a model (adapted from Chapman et al., 2003). Units are µg/g dry weight of *Ephoron virgo* nymphs, eggs and adults (males and females). OCs: organochlorine compounds.

The lower concentrations in eggs compared to nymphs and adults in most of the pollutants could be a key factor to explain the population success of *E. virgo* at sites far from the sediment dump. Although bioaccumulation problems at these locations exist, nymphs could suffer sublethal effects affecting reproduction (Conley et al., 2009). However, it did not seem to be the case in the lower Ebro due to the high numbers of emergent adults with high proportions of females reported in **Chapter 3**. As explained in **Chapter 5**, the low OC transfer to eggs could be linked to the lipid content and composition of eggs, by the mechanisms of lipid transport and by the structure of the eggshell. These factors could make possible that the pollutant transfer to the following generation of nymphs was lower, although the effect on the hatching success or on the new offspring development was not studied.

The different bioaccumulation patterns along nymph growth depending on the compound analyzed of *E. virgo* may be related with the size of particle ingested, the hydraulic preferences at each instar, and to the amount of polluted sediments released from the Flix dam according to flow. The bioaccumulation patterns of the most

persistent compounds from Chapters 4 and 5 are shown in Figure 11, combining the weight measures of E. virgo populations at each instar from Chapter 3 in order to estimate the contribution of Hg, Cd, DDEs, DDTs and PCBs to the benthic compartment by this species. The highest persistence of those pollutants in the benthos ranged from May to July, when emergences began and, as shown in Figure 10, most of the metals and OCs fate was transferred to the adults. One of the reasons for the low concentration of pollutants in larger nymphs collected in the beginning of August could be that they move to slow flowing areas in order to emerge and therefore reflect the bioavailable pollutants in slow flowing areas, which can be lower due to a lower flow exposure. Therefore, the hydraulic preferences of macroinvertebrates and the flow patterns of the river are determinnant for the knowledge of the bioaccumulation dynamics under field conditions. In **Chapter 2**, species inhabiting areas of high flow were mostly filter-feeders or omnivorous species such as Orthocladiinae, which could be indicators of the bioavailable pollutant loading carried by flow. On the other hand, species living in slow- flowing areas such as deposit feeders (e.g., *Caenis luctuosa*) could reflect the bioavailability of pollutants in depositional areas of the river. This point of view could be helpful to understand the pollution patterns in the whole community at mesohabitat scale.

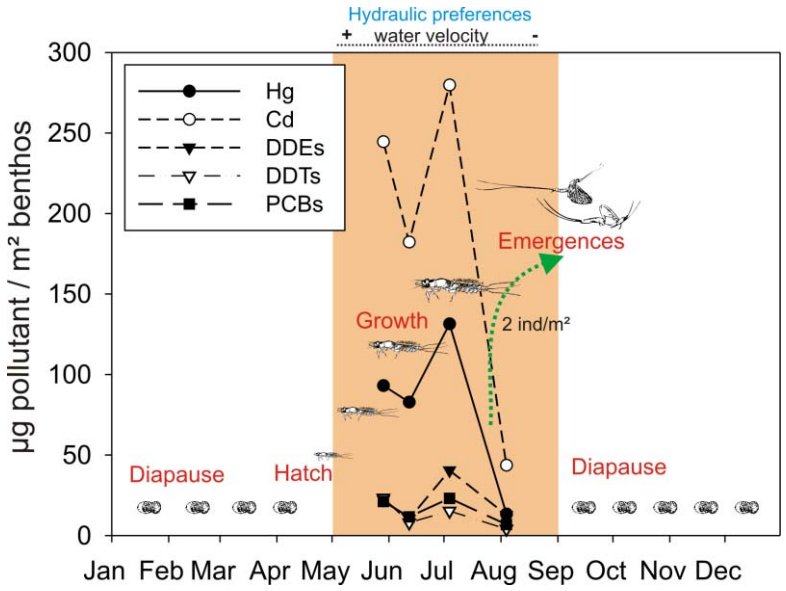


Figure 11. Pollutant contribution per m^2 to the benthic compartment by the bioaccumulation in *E. virgo* at Tortosa, 65 km downstream of the sediment dump. Hydraulic preferences based on Sagnes et al., (2008). Data calculations were extracted from Chapters 3, 4 and 5.

Additionally, since bioaccumulation of pollutants in marine filter feeders is temperature-dependant (e.g., Odin et al., 1997; Loayza-Muro & Elías-Letts, 2007), if global temperature increases (IPCC, 2007) the pollutant uptake could increase due to higher filtration rates and changes in solution chemistry and physical kinetics (Mubiana & Blust, 2007). Thus, apart from alterations on the phenology of species (e.g., *E. virgo* life cycle advance of almost a month in 2005 compared to 1987 **in Chapter 3**), changes as higher bioaccumulation rates and its toxicological responses could occur.

On the other hand, results of the present thesis regarding the ecological risk assessment in the lower Ebro River showed that metal and organochlorine compound exposures were caused by historical pollution from the sediments in the Flix reservoir which chronically impairs the aquatic fauna, mostly reflected in pollutant sensitive species and in changes of the function and structure of the community. A recovery of pollutant-sensitive species such as *E. virgo* at sites where metal and OC exposures was demonstrated to be higher would be indicative of remediation. Since a restoration plan consisting in sediment removal of the Flix reservoir is in process (Resolución de la Confederación Hidrogràfica del Ebro, 2006), and the European Directive 2006/11/CE concering dangerous substances into the aquatic environment has to be implemented, changes in populations of pollutant sensitive species and a reduction of the bioaccumulation levels in the lower Ebro ecosystem should be expected.

Since many other environmental factors could be also determinant for the macroinvertebrate composition (flow events, oxygen, nutrients, presence of macrophtes and other habitat constraints) it is difficult to separate the other impacts from metal and organochlorine pollution. However, the present thesis provided a strong evidence of the ecological response to the anthropogenic impacts that the lower Ebro River is suffering, and demonstrated that these effects acted at different levels of organization, including communities, populations and individuals.

References

Araujo, R. & Ramos, M.A. (2000) Status and conservation of the giant European freshwater pearl mussel (*Margaritifera auricularia*) (Spengler, 1793) (Bivalvia : Unionoidea). *Biological Conservation,* **96,** 233-239.

Archaimbault, V., Usseglio-Polatera, P., Garric, J., Wasson, J.G. & Babut, M. (2010) Assessing pollution of toxic sediment in streams using bio-ecological traits of benthic macroinvertebrates. *Freshwater Biology,* **55,** 1430-1446.

Bady, P., Dolédec, S., Fesl, C., Gayraud, S., Bacchi, M. & Scholl, F. (2005) Use of invertebrate traits for the biomonitoring of European large rivers: the effects of sampling effort on genus richness and functional diversity. *Freshwater Biology,* **50,** 159-173.

Bartrons, M., Grimalt, J.O. & Catalán, J. (2007) Concentration changes of organochlorine compounds and polybromodiphenyl ethers during metamorphosis of aquatic insects. *Environmental Science & Technology,* **41,** 6137-6141.

Bêche, L.A. & Statzner, B. (2009) Richness gradients of stream invertebrates across the USA: taxonomy- and trait-based approaches. *Biodiversity and Conservation,* **18,** 3909-3930.

Bonada, N., Dolédec, S. & Statzner, B. (2007) Taxonomic and biological trait differences of stream macroinvertebrate communities between mediterranean and temperate regions: implications for future climatic scenarios. *Global Change Biology,* **13,** 1658-1671.

Buchwalter, D.B., Cain, D.J., Martin, C.A., Xie, L., Luoma, S.N. & Garland, T., Jr. (2008) Aquatic insect ecophysiological traits reveal phylogenetically based differences in dissolved cadmium susceptibility. *Proceedings of the National Academy of Sciences of the United States of America,* **105,** 8321-8326.

Cain, D.J., Luoma, S.N., Carter, J.L. & Fend, S.V. (1992) Aquatic Insects as Bioindicators of Trace-Element Contamination in Cobble-Bottom Rivers and Streams. *Canadian Journal of Fisheries and Aquatic Sciences,* **49,** 2141-2154.

Cain, D.J., Luoma, S.N. & Wallace, W.G. (2004) Linking metal bioaccumulation of aquatic insects to their distribution patterns in a mining-impacted river. *Environmental Toxicology and Chemistry,* **23,** 1463-1473.

Chapman, P.M., Wang, F.Y., Janssen, C.R., Goulet, R.R. & Kamunde, C.N. (2003) Conducting ecological risk assessments of inorganic metals and metalloids: Current status. *Human and Ecological Risk Assessment,* **9,** 641-697.

CHE (2005) Evaluación de los impactos del cambio climático en los recursos hídricos del río Ebro. Available at http://www.chebro.es/contenido.visualizar.do?idContenido=14281&idMenu=3084

Clements, W.H. (1999) Metal tolerance and predator-prey interactions in benthic macroinvertebrate stream communities. *Ecological Applications,* **9,** 1073-1084.

Clements, W.H., Carlisle, D.M., Lazorchak, J.M. & Johnson, P.C. (2000) Heavy metals structure benthic communities in Colorado mountain streams. *Ecological Applications,* **10,** 626-638.

Conley, J.M., Funk, D.H. & Buchwalter, D.B. (2009) Selenium Bioaccumulation and Maternal Transfer in the Mayfly *Centroptilum triangulifer* in a Life-Cycle, Periphyton-Biofilm Trophic Assay. *Environmental Science & Technology,* **43,** 7952-7957.

Corkum, L.D., Ciborowski, J.J.H. & Lazar, R. (1997) The distribution and contaminant burdens of adults of the burrowing mayfly, *Hexagenia*, in Lake Erie. *Journal of Great Lakes Research,* **23,** 383-390.

De Crespin De Billy, V. & Usseglio-Polatera, P. (2002) Traits of brown trout prey in relation to habitat characteristics and benthic invertebrate communities. *Journal of Fish Biology,* **60,** 687-714.

De Haas, E.M., Reuvers, B., Moermond, C.T.A., Koelmans, A.A. & Kraak, M.H.S. (2002) Responses of benthic invertebrates to combined toxicant and food input in floodplain lake sediments. *Environmental Toxicology and Chemistry,* **21,** 2165-2171.

De Lange, H.J., De Jonge, J., Den Besten, P.J., Oosterbaan, J. & Peeters, E. (2004) Sediment pollution and predation affect structure and production of benthic macroinvertebrate communities in the Rhine-Meuse delta, The Netherlands. *Journal of the North American Benthological Society,* **23,** 557-579.

Directive 2006/11/EC of the European Parliament and of the Council of 15 February 2006 on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community. Official Journal 4 March 2006 L 64/52. European Commission, Brussels.

Dolédec, S., Lamouroux, N., Fuchs, U. & Merigoux, S. (2007) Modelling the hydraulic preferences of benthic macroinvertebrates in small European streams. *Freshwater Biology,* **52,** 145-164.

Dolédec, S. & Statzner, B. (2008) Invertebrate traits for the biomonitoring of large European rivers: an assessment of specific types of human impact. *Freshwater Biology,* **53,** 617-634.

Dunbar, M.J., Pedersen, M.L., Cadman, D., Extence, C., Waddingham, J., Chadd, R. & Larsen, S.E. (2010) River discharge and local-scale physical habitat influence macroinvertebrate LIFE scores. *Freshwater Biology,* **55,** 226-242.

Edsall, T.A., Manny, B.A., Schloesser, D.W., Nichols, S.J. & Frank, A.M. (1991) Production of *Hexagenia limbata* in contaminated sediments in the upper Great Lakes connecting channels. *Hydrobiologia,* **219,** 353-361.

European Commission. 2000. Directive 2000/60/EC of the European Parliament and of the Council. Establishing a framework for Community action in the field of water policy, Brussels, Belgium.

Gordo, O. & Sanz, J.J. (2005) Phenology and climate change: a long-term study in a Mediterranean locality. *Oecologia,* **146,** 484-495.

Gore, J.A. (1978) Technique for predicting in-stream flow requirements of benthic macroinvertebrates. *Freshwater Biology,* **8,** 141-151.

Gore, J.A., Layzer, J.B. & Mead, J. (2001) Macroinvertebrate instream flow studies after 20 years: A role in stream management and restoration. *Regulated Rivers-Research & Management,* **17,** 527-542.

Horrigan, N. & Baird, D.J. (2008) Trait patterns of aquatic insects across gradients of flow-related factors: a multivariate analysis of Canadian national data. *Canadian Journal of Fisheries and Aquatic Sciences,* **65,** 670-680.

Hutchens, J.J., Chung, K. & Wallace, J.B. (1998) Temporal variability of stream macroinvertebrate abundance and biomass following pesticide disturbance. *Journal of the North American Benthological Society,* **17,** 518-534.

IPCC (2007) Climate change 2007: impacts, adaptation and vulnerability. Summary for policymakers, p 22.

James, A.B.W. & Suren, A.M. (2009) The response of invertebrates to a gradient of flow reduction - an instream channel study in a New Zealand lowland river. *Freshwater Biology,* **54,** 2225-2242.

Klok, C. & Kraak, M.H.S. (2008) Living in highly dynamic polluted river floodplains, do contaminants contribute to population and community effects? *Science of the Total Environment,* **406,** 455-461.

Kureck, A. & Fontes, R.J. (1996) The life cycle and emergence of *Ephoron virgo*, a large potamal mayfly that has returned to the River Rhine. *Archiv für Hydrobiologie Supplement,* **113,** 319-323.

Lamouroux, N., Dolédec, S. & Gayraud, S. (2004) Biological traits of stream macroinvertebrate communities: effects of microhabitat, reach, and basin filters. *Journal of the North American Benthological Society,* **23,** 449-466.

Liess, M. & Von Der Ohe, P.C. (2005) Analyzing effects of pesticides on invertebrate communities in streams. *Environmental Toxicology and Chemistry,* **24,** 954-965.

Loayza-Muro, R. & Elias-Letts, R. (2007) Responses of the mussel *Anodontites trapesialis* (Unionidae) to environmental stressors: Effect of pH, temperature and metals on filtration rate. *Environmental Pollution,* **149,** 209-215.

Maret, T.R., Cain, D.J., Maccoy, D.E. & Short, T.M. (2003) Response of benthic invertebrate assemblages to metal exposure and bioaccumulation associated with hard-rock mining in northwestern streams, USA. *Journal of the North American Benthological Society,* **22,** 598-620.

Merigoux, S. & Dolédec, S. (2004) Hydraulic requirements of stream communities: a case study on invertebrates. *Freshwater Biology,* **49,** 600-613.

Mubiana, V.K. & Blust, R. (2007) Effects of temperature on scope for growth and accumulation of Cd, Co, Cu and Pb by the marine bivalve *Mytilus edulis*. *Marine Environmental Research,* **63,** 219-235.

Odin, M., Ribeyre, F. & Boudou, A. (1997) Depuration processes after exposure of burrowing mayfly nymphs (*Hexagenia rigida*) to methylmercury and cadmium from water column or sediment: Effects of temperature and pH. *Aquatic Toxicology,* **37,** 125-137.

Peru, N. & Dolédec, S. (2010) From compositional to functional biodiversity metrics in bioassessment: A case study using stream macroinvertebrate communities. *Ecological Indicators,* **10,** 1025-1036.

Poff, N.L. & Ward, J.V. (1990) Physical habitat remplate of lotic systems. Recovery in the context of historical pattern of spatiotemporal heterogeneity. *Environmental Management,* **14,** 629-645.

Poff, N.L. & Zimmerman, J.K.H. (2010) Ecological responses to altered flow regimes: a literature review to inform the science and management of environmental flows. *Freshwater Biology,* **55,** 194-205.

Ramos, M.A. (1998) Implementing the Habitats Directive for mollusc species in Spain. *Journal of Conchology*, special publication, **2**, 125-132.

Resolución de la Confederación Hidrográfica del Ebro por la que se anuncia: Información Pública del Proyecto Informativo de la eliminación de la contaminación química del embalse de Flix y su estudio de impacto ambiental. Boletín Oficial del Estado (2 febrero 2006) 28, 1069.

Sánchez, R. & Ibáñez, C. Agència Catalana de l'Aigua. Available at http://acaweb.gencat.cat/aca/documents/ca/planificacio/cabals/cabals_ambientals_baix_ebre_2008.pdf.

Schulz, R. & Dabrowski, J.M. (2001) Combined effects of predatory fish and sublethal pesticide contamination on the behavior and mortality of mayfly nymphs. *Environmental Toxicology and Chemistry,* **20,** 2537-2543.

Smits, J.E.G., Bortolotti, G.R., Sebastian, M. & Ciborowski, J.J.H. (2005) Spatial, temporal, and dietary determinants of organic contaminants in nestling tree swallows in Point Pelee National Park, Ontario, Canada. *Environmental Toxicology and Chemistry,* **24,** 3159-3165.

Smock, L.A. (1983) Relationships between Metal Concentrations and Organism Size in Aquatic Insects. *Freshwater Biology,* **13,** 313-321.

Snook, D.L. & Milner, A.M. (2002) Biological traits of macroinvertebrates and hydraulic conditions in a glacier-fed catchment (French Pyrenees). *Archiv Fur Hydrobiologie,* **153,** 245-271.

Southwood, T.R.E. (1988) Tactics, strategies and templets. *Oikos,* **52,** 3-18.

Standley, L.J., Sweeney, B.W. & Funk, D.H. (1994) Maternal transfer of chlordane and its metabolites to the eggs of a stream mayfly *Centroptilum triangulifer*. *Environmental Science & Technology,* **28,** 2105-2111.

Statzner, B. & Bêche, L.A. (2010) Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? *Freshwater Biology,* **55,** 80-119.

Suren, A.M. & Jowett, I.G. (2006) Effects of floods versus low flows on invertebrates in a New Zealand gravel-bed river. *Freshwater Biology,* **51,** 2207-2227.

Tachet, H., Ricoux, P., Bournaud, M. & Usseglio- Polatera, P. (2000) Invertebrés d'eau douce: systématique, biologie, écologie., CNRS Editions, Paris.

Tomanova, S. & Usseglio-Polatera, P. (2007) Patterns of benthic community traits in neotropical streams: relationship to mesoscale spatial variability. *Fundamental and Applied Limnology,* **170,** 243-255.

Townsend, C.R. & Hildrew, A.G. (1994) Species traits in relation to a habitat templet for river systems. *Freshwater Biology,* **31,** 265-275.

Conclusions

The main conclusions of the thesis answering the questions of the objectives are the following:

1. At community level, the taxonomic-based approach indicated a different community composition as the river distance increased downstream of the main impacts (dams and polluted sediments). These changes influenced the taxonomy-based community metrics, with higher values at the sites located more downstream due to the presence of species sensitive to chemical pollution.

2. The temporal variability according to different hydrological conditions was mainly reflected by the functional-based approach (biological trait composition and its functional metrics), while slight changes in taxonomic metrics were detected.

3. Within all the habitat constraints measured at mesohabitat scale, direct measurements as the water velocity and Froude and Reynolds numbers explained most of the variability of the benthic macroinvertebrate assemblages and of their functional traits. However, the cover of macrophytes on the benthos and the interstitial dissolved oxygen did not appear to be determinant.

4. When measuring the ecological niche of species, those taxa with a high marginality occupied narrower habitat conditions (slow and fast flowing areas) while other species were tolerant to a wider range of conditions.

5. The taxonomic resolution was crucial to obtain unequivocal interpretations of the community patterns along the hydraulic gradient, with special attention to

6. the group of Chironomidae, wich presented different habitat preferences within the same subfamily, tribe or genus. Consequently, the taxonomic resolution influenced the response of the community metrics to hydraulics, since highly diverse Chironomidae increased the power of the analysis.

7. Most of the functional characteristics of macroinvertebrates (biological traits) responded positively or negatively to the hydraulic conditions (e.g. feeding, locomotion) as a result of an adaptation to the environmental conditions, while other traits related with life cycle could reflect adaptations to flow regime events or species interactions.

8. At population level, an increase of 2ºC in the mean daily air temperature during growth period of the keystone species *Ephoron virgo* compared to 1987, toghether with a higher value of degree days accumulated in 2005, appeared to be the main reason for the advance of 3 weeeks of its life cycle.

9. Secondary production estimates of *E. virgo* after the establishment of alien species with same feeding habits (*Dreissena polymorpha* and *Corbicula fluminea*) showed higher values in 2005 than in 1987, probably due to the dramatic decline of native freshwater mussels which filtration activity could have been replaced by those alien species.

10. The foodweb of the lower Ebro River presented important problems of bioaccumulation of toxic substances mainly originated in the sediment dump at the Flix reservoir. A wide variety of pollutants, including heavy metals and organochlorine compounds, are bioaccumulated at high levels by populations of filter feeding macroinvertebrates downstream of the pollution source.

11. Different patterns of bioaccumulation were observed along the gowth of *E. virgo* and along the stages of its life cycle. Nymphs and emergent adults presented the highest concentrations of metals and organochlorine compounds, representing a high risk to the transfer of pollutants to higher trophic levels. The maternal transfer of pollutants to eggs existed, although was lower compared to those pollutant concentrations in adult females.

12. The different compounds analyzed presented metal- and organochlorine-specific bioaccumulation patterns, mainly attributed to the variable bioavailability in the

river at every moment depending on the flow, and to the affinity of each compound to organic materials.

13. Interespecific differences in bioaccumulation were observed, with higher levels of pollutants in the sensitive species *E. virgo* compared to *H. exocellata* mainly due to their ecophysiological traits.

Overall, the response to the different type of stressors can be complex, moreover when including pollution by heavy metals and organochlorine compounds combined with flow alterations. However, since only those species relatively sensitive to chemical pollution contribute to the detection of changes at community level, we demonstrated that *E. virgo* is a good bioindicator of the ecological risk by toxic contaminants in large rivers as the Ebro. Moreover, this organism has been already used in laboratory tests, incrementing their applicability in other levels of organization (e.g., use of biomarkers).

On the other hand, the knowledge of the response of the macroinvertebrate community and its functional structure to the hydraulic conditions can have potential applications in the guiding management of environmental flows in the lower Ebro River by providing useful species-specific information for the application of habitat models using macroinvertebrates.