

FLOW ALTERATION AND WASTEWATER INPUTS EFFECTS ON FRESHWATER COMMUNITIES IN MEDITERRANEAN RIVERS

Jordi-René Mor Roy

Per citar o enllaçar aquest document:

Para citar o enlazar este documento:

Use this url to cite or link to this publication:

<http://hdl.handle.net/10803/668849>

ADVERTIMENT. L'accés als continguts d'aquesta tesi doctoral i la seva utilització ha de respectar els drets de la persona autora. Pot ser utilitzada per a consulta o estudi personal, així com en activitats o materials d'investigació i docència en els termes establerts a l'art. 32 del Text Refós de la Llei de Propietat Intel·lectual (RDL 1/1996). Per altres utilitzacions es requereix l'autorització prèvia i expressa de la persona autora. En qualsevol cas, en la utilització dels seus continguts caldrà indicar de forma clara el nom i cognoms de la persona autora i el títol de la tesi doctoral. No s'autoritza la seva reproducció o altres formes d'explotació efectuades amb finalitats de lucre ni la seva comunicació pública des d'un lloc aliè al servei TDX. Tampoc s'autoritza la presentació del seu contingut en una finestra o marc aliè a TDX (framing). Aquesta reserva de drets afecta tant als continguts de la tesi com als seus resums i índexs.

ADVERTENCIA. El acceso a los contenidos de esta tesis doctoral y su utilización debe respetar los derechos de la persona autora. Puede ser utilizada para consulta o estudio personal, así como en actividades o materiales de investigación y docencia en los términos establecidos en el art. 32 del Texto Refundido de la Ley de Propiedad Intelectual (RDL 1/1996). Para otros usos se requiere la autorización previa y expresa de la persona autora. En cualquier caso, en la utilización de sus contenidos se deberá indicar de forma clara el nombre y apellidos de la persona autora y el título de la tesis doctoral. No se autoriza su reproducción u otras formas de explotación efectuadas con fines lucrativos ni su comunicación pública desde un sitio ajeno al servicio TDR. Tampoco se autoriza la presentación de su contenido en una ventana o marco ajeno a TDR (framing). Esta reserva de derechos afecta tanto al contenido de la tesis como a sus resúmenes e índices.

WARNING. Access to the contents of this doctoral thesis and its use must respect the rights of the author. It can be used for reference or private study, as well as research and learning activities or materials in the terms established by the 32nd article of the Spanish Consolidated Copyright Act (RDL 1/1996). Express and previous authorization of the author is required for any other uses. In any case, when using its content, full name of the author and title of the thesis must be clearly indicated. Reproduction or other forms of for profit use or public communication from outside TDX service is not allowed. Presentation of its content in a window or frame external to TDX (framing) is not authorized either. These rights affect both the content of the thesis and its abstracts and indexes.

Flow alteration and wastewater inputs effects on freshwater communities in Mediterranean rivers

Jordi-René Mor Roy
Doctoral Thesis

FLOW ALTERATION AND WASTEWATER INPUTS EFFECTS ON FRESHWATER COMMUNITIES IN MEDITERRANEAN RIVERS



Universitat
de Girona

Jordi-René Mor Roy
Doctoral Thesis
2019

ICRA
Institut Català
de Recerca de l'Aigua



Doctoral Thesis

Flow alteration and
wastewater inputs effects on
freshwater communities
in Mediterranean rivers

Jordi René Mor Roy

2019



Doctoral Thesis

Flow alteration and wastewater inputs
effects on freshwater communities
in Mediterranean rivers

Jordi René Mor Roy

2019

Doctoral program in Water Science and Technology

Supervised by:

Sergi Sabater i Cortés (Universitat de Girona, Institut Català de Recerca de l'Aigua)

Isabel Muñoz Gracia (Universitat de Barcelona)

Tutored by:

Sergi Sabater i Cortés (Universitat de Girona, Institut Català de Recerca de l'Aigua)

Thesis submitted in fulfillment of the requirements for the doctoral degree at the University of Girona.



Dr. Sergi Sabater i Cortés of the University of Girona
and Dr. Isabel Muñoz Gracia of the University of
Barcelona,

DECLARE:

That the thesis titles “*Flow alteration and wastewater inputs effects on freshwater communities in Mediterranean rivers*”, presented by Jordi René Mor Roy to obtain a doctoral degree, has been completed under our supervision and meets the requirements to opt for an *International Doctorate*.

For all intents and purposes, we hereby sign this document.

Dr. Sergi Sabater i Cortés

Dr. Isabel Muñoz Gracia

Girona, 2019

“Pare
Digueu-me què
Li han fet al riu
Que ja no canta
Rellisca com un barb
Mort sota un pam
D'escuma blanca
Pare
El riu ja no és el riu
Pare
Abans que torni l'estiu
Amagui tot el que és viu”

Pare – J.M Serrat
(a la nostra generació popularitzada per SAU)

Al meu pare,

Acknowledgements (*and apologies*)

Aquells que em coneixeu de fa anys sou probablement els únics conscients de l'esforç que ha representat poder començar a fer aquesta tesi. Sense exagerar, va ser major que fer-la, i malgrat que normalment s'agraeix a qui ha ajudat al desenvolupament del doctorat, aquest no hauria estat possible sense tots els ànims i cops de mà rebuts amb anterioritat.

Deixeu-me però començar per els meus directors de tesi. Voldria agrair a en Sergi i a la Isabel l'oportunitat que em varen lliurar. Recordaré (i li recordaré) sempre quan cert professor em va veure obrint estómacs de peix i em va dir que allò era una “*guarrada*”. Uns anys més tard, vaig acabar treballant per ell i...bé, llegiu la tesi. Seriosament, gràcies, gràcies per les infinites correccions, el detallisme a l'hora de mirar els treballs, la llibertat que m'heu donat, la manca de límits per tot el que se'm passava pel cap, la vostra humanitat, suport i sobretot, paciència. En tot moment heu esperat que jo veies la llum a cada article quan feia mesos que vosaltres ja la hi veieu. Em sap greu haver estat fora tant de temps, i més en aquesta part final de la tesi, i també, voldria disculpar-me per les preses a l'hora d'acabar...fa temps que no us ho dic però...”*Moltes gràcies i perdoneu les molèsties*”.

Voldria començar agraint a tots els que sense la vostra intervenció clau, jo hauria pres una altra direcció. Vaig estar forces anys

intentant trobar l'oportunitat de fer una tesi pagada, i vaig aprendre moltes maneres de ni aconseguir una feina ni una beca. Papa, de tu he après tot el que he pogut. Segurament, mai tindrè la feina que tu hauries pretès, amb els horaris que em desitjaries i probablement, ni tant sols, al país on em voldries. Però tot i així, sempre has estat al meu costat i t'has sacrificat més que cap altra persona. Moltíssimes gràcies!. Coral, moltes gràcies per ser-hi en aquells moments de desànim, donar-me tot el suport del món i no deixar que em rendís. Entrant en el terreny professional, vull agrair els professors que em varen acollir, ensenyar les beceroles de la recerca i tantes altres coses; Lluís Z., Dani B. i Jordi S., heu estat tres pilars que sempre teniu temps per als estudiants. Em sap greu tindre la sensació que mai us ho podré agrair prou. No puc contar les hores que de manera completament altruista i vocacional ens heu dedicat als estudiants de la facultat que volíem fer recerca, moltes gràcies per la tasca que feu. De l'etapa UdG (això costarà perquè he vist un parell de generacions...) vull agrair a tothom el seu temps i sobretot les estones de laboratori+clàssica/jazz/Tom Waits/ràdio amb els Jordis (mitjà i gran) i gràcies per les divagacions a l'hora del cafè o mostrejant a tothora: Núria A., Jordi C., Miquel J., Joan N., Irene T., Laura B., Laura D... I també, agrair a l'Anna R. i a la Helena G. la feina feta al capdavant del programa de doctorat.

A l'ICRA vaig arribar amagat, com no pot ser menys en mi, en un cau (L23-“fonsu”), i allà vaig començar a expandir-me i anar-vos coneixent a tots. Moltes gràcies: Lydia (per passar-me els taps dels pots de caramel); Elisabet (per els moments de safareig que tan bé

sabem fer); Gemma U. (encara tinc un taka-taka guardat per si vols anar a córrer); el grup “retro” del D29: Gemma, Cristina, Joan Pere...us he trobat molt a faltar, gràcies per fer que fos divertit anar a treballar...JP, sempre recordaré els nostres cafès negres i la pissarra. I també, a totes les altres que vàreu arribar després (si més no al meu radi): Ada, Núria, Anna (us estimo molt! Gràcies per la vostra amistat!); Carmen (gracias por toda la ayuda en el laboratorio y preparando material a toda prisa! “eres un suelo!”), Julio (Gràcies per els consells!), Rafa, Vicenç, Marta T., Laurie B., Laura V., Maria C., Míriam, Ferran, Mireia, Carme, Dídac, Albert H., Eduardo Z. (“*Qué bueno que viniste!*”, y que paciencia llevarte a campo!)...I també les companyes de la UB: Rebeca, Sílvia, Núria, Meritxell i Marc...A totes, Moltes gràcies!!

Del icra he sacado buenas amistades, que crecieron cuando compartíamos despacho ¡y casa! Juan David, gracias por tus mensajes, amistad y... haberte ido a Colombia en el momento idóneo! Daniel, eres una persona maravillosa, he tenido mucha suerte de haberte conocido, gracias por todo! Nell'appartamento sono anche passati Vuong (Cám ơn bạn đã dành thời gian lắng nghe và chia sẻ, uống trà cùng tôi) e Arianna...surts més endavant.

Moltes gràcies Albert R. i Vicenç per fer el paper de “germanets” grans. Molts punts d’aquesta tesi no s’haurien desencallat sense els vostres consells, ànims i...com a bons germans, estirades d’orella. Com a “bon” germanet petit he fet la meva molts cops...em sap greu, però moltes gràcies per la paciència i el temps que m’heu dedicat!

Al grupo de Euskadi, Olatz, Arturo y Dani; mila esker landan zein tesiko etapa desberdinetan eman didazuen laguntzarengatik. Zuei esker “*esta mierda de rios que tenéis en cataluña*” asko gozatu dut. Ezin dut ekidin erreka hauek hautatu izanagatik pixka bat errudun sentitzea, baina gauza onak irten dira. Pentsatuz gero, seguru baten bat bururatzeko zaigula, ezta? Olatz, zor dizkidazun 1000 garagardoak sagardotegi batean egun bat emateagatik aldatzea onartzen dut ;) Espero dut zuri ere dena ondo joatea eta elkarren arteko kontaktua mantentzea. Zurekin lanean dihardutea plazer bat izan da. Eskerrik asko Ioar, testua posible egiteko!

Sylvain D., je vous remercie pour votre accueil et pour me montrer les rivières du massif central, la délicieuse nourriture pendant les travaux sur le terrain... le vin, et tout ce que l'on peut considérer comme des petits plaisirs de la recherche. Merci à tous les collègues du couloir et à votre magnifique tradition d'une bouteille de vin pour chaque papier. Et, Charlene! Merci beaucoup pour être une très bonne amie, pour toutes nos conversations « Yogi Tea » et aussi pour me faire avoir un peu de vie sociale à Lyon.

To the Berkeley people; I've no words. Sarah and Syd thank you for opening the doors of your home and let me feel in family. Thanks Syd for give me the opportunity to know California and your lovely Sierra (both mountain and beer!) and always...*Go Giants!! and buuuuhh Dodgers!!!* Stephany C. thank you to involve me inside the department as a normal member and thanks to all of you: Albert, Núria, Ross, Susanne, Hanna, Liss, Brian, Pablo, Celso, Maria... for these great months!

E infine, ma solo perché è in ordine cronologico, alla gente di Pavia: Elisa S., Enrico, Zeno, Fra, Alice and Elisa C. Grazie mille per il benvenuto, le birre e l'aiuto nella parte finale di questa tesi. I a tu Pere, que no sabia on encabir-te! Moltes, moltíssimes gràcies per ser-hi sempre. Gràcies per els ànims, les cerveses pensant projectes nous (que potser mai farem, però hem rigut molt); dir que l'Arianna és una *foca*, i tantíssimes altres coses. Merci!

Tornant al terreny més personal, Rous, gràcies per la teva amistat i per ensenyar-me que no puc dedicar "100mil hores" a la feina. Potser això fa que hagi de demanar perdó, altre cop, als meus directors per tindre la sensació que podria haver fet més del que he fet, però tenies raó al dir que, o feia aquesta feina tinguent vida o millor canviar de feina. I per últim, el contrast, Arianna. Grazie per esserti presentata subito per come sei, con l'energia e il sorriso che ti caratterizzano. Mi hai reso felice e hai lavorato come nessuno. Grazie per il tuo "*Non andiamo fino a che non finisci!*". Come dice Dani, sei un po' una "WorkAholiC", ma lo apprezzo davvero ... e niente ... ora tocca te, tutto ancora è abbastanza calmo ... Ti guiderò io, non ti lascerò mai allontanare dal PC! Nessuna paura!

A tots, Moltes Gràcies!

Derived Scientific Publications

Mor, J.R., A. Ruhí, E. Tornés, H. Valcárcel, I. Muñoz and S. Sabater. 2018. Dam regulation and riverine food-web structure in a Mediterranean river. *Science of the Total Environment*. **625**:301-310.

Mor, J.R., S. Dolédec, V. Acuña, S. Sabater and I. Muñoz. Invertebrate community responses to urban wastewater effluent pollution under different hydro-morphological conditions. Under revision in *Environmental Pollution*. **252**: 483-492.

Mor, J.R., I. Muñoz, S. Sabater, LL. Zamora and A. Ruhí. The silent predator: effects of urban wastewater on community size structure and energy transfer efficiency through river food webs. *In Prep.*

List of acronyms

α -diversity	Alpha-diversity
AFDW	Ash free dry weight
ANOVA	Analysis of variance
β -diversity	Beta-diversity
CBOM	Coarse benthic organic matter
CdS	Cobble-dominated streams
CSS	Community size spectrum
CWMBS	Community-weighted mean body size
D1 (Chapter 1)	Site downstream 1
D2 (Chapter 1)	Site downstream 2
D3 (Chapter 1)	Site downstream 3
D ₅₀ grain size	Median (50%) grain diameter
DOC	Dissolved organic carbon analyses
EC ₅₀	Effect concentration for 50% of individuals
EPT	Ephemeroptera, Plecoptera and Trichoptera
EU	European Union
FBOM	Fine benthic organic matter
FCL	Food-chain length
γ -diversity	Gamma-diversity
GF/F filters	Glass microfiber filters
HSD test	Honestly-significant-difference test
Ht	Tendency to lose or gain water
LMM	Linear mixed models
LTWD	Long-term water level differences
NH ₄ ⁺	Ammonium
NO ₂ ⁻	Nitrite
NO ₃ ⁻	Nitrate
PCA	Principal component analysis
PERANOVA	Permutational analysis of variance
PERMANOVA	Permutational multivariate analysis of variance

Phacs	Pharmaceutical products
PO_4^3	Phosphate
SdS	Sand-dominated streams
SEAb	Standard ellipse area estimated via Bayesian inference
SIA	Stable isotope analyses
SIMPER	Similarity percentages
SMA slope	Standardised major axis slope
U1 (Chapter 1)	Site upstream

List of figures

GENERAL INTRODUCTION

Fig. I1 River inputs and outputs of water and matter	42
Fig. I2 River food web diagram	44
Fig. I3 Stream typologies on Mediterranean regions.....	49

AIMS

Fig. A1 Principal questions covered in the thesis	59
---	----

GENERAL METHODS

Fig. M1 Study sites map.....	70
------------------------------	----

CHAPTER 1

Fig. 1.1 Locations of the study sites in the Montsant River.....	80
Fig. 1.2 Macroinvertebrate feeding strategies at each study site	92
Fig. 1.3 Macroinvertebrate body size distribution at the different study sites	97
Fig. 1.4 Food-web diagrams and metrics	99

CHAPTER 2

Fig. 2.1 Location of the studied streams	110
Fig. 2.2 Shannon taxonomic alpha diversity	129
Fig. 2.3 Comparison of the relative utilization of each trait category among dry and wet periods	133

CHAPTER 3

Fig. 3.1 Wastewater effects on food web energy transfer theoretical framework.....	144
--	-----

Fig. 3.2 Predators and wastewater pollution.....	157
Fig. 3.3 Wastewater pollution effects on energy efficiency transfer.....	159
Fig. 3.4 Wastewater pollution impacts on trophic niche area	162
Fig. 3.5 Predator-Consumers trophic niche interactions	164

List of tables

GENERAL METHODS

Table M1 Summary of the methods used	63
Table M2 Environmental characteristics of the studied sites.....	71

CHAPTER 1

Table 1.1 Hydromorphological and water variables	88
Table 1.2 Stream relative cover proportion	89
Table 1.3 Coarse benthic organic matter (CBOM), fine benthic organic matter (FBOM), algae, bryophyte, and macrophyte biomass).....	89
Table 1.4 Invertebrate and vertebrate community structure.....	94
Table 1.5 Food-web structure metrics	98

CHAPTER 2

Table 2.1 Studied sites description	112
Table 2.2 Studied sites environmental conditions.....	120
Table 2.3 Hydrological, nutrients and pharmaceutical	123
Table 2.4 Mean macroinvertebrate richness, Ephemeroptera, Plecoptera and Trichoptera (EPT) richness, and diversity.	124
Table 2.5 Macroinvertebrate SIMPER analysis results	127
Table 2.6 Wastewater effects on traits categories.....	131

CHAPTER 3

Table 3.1 Upstream (Up) and downstream site (Down) nutrient and pharmaceutical concentrations on spring 2016.....	155
---	-----

Index

Summary	28
Resum.....	31
Resumen.....	35
General Introduction	40
Rivers and who lives in them.....	42
Mediterranean rivers: characteristics and anthropogenic pressures.....	46
Why do we care about rivers?	48
Aims.....	56
General Methods	60
Thesis Chapters.....	73
Chapter 1	74
Background.....	76
Material and Methods	79
Results	87
Discussion.....	96
Chapter 2	106
Background.....	108
Material and Methods	110
Results	119

Discussion.....	134
Chapter 3	140
Background.....	142
Material and Methods.....	145
Results.....	152
Discussion.....	160
General Discussion.....	168
Anthropogenic impacts lead to stream community's homogenization? The community contribution.....	171
Anthropogenic impacts change ecosystem boundaries.....	174
Learning from the thesis: Implications for future research.	175
Conclusions.....	182
References.....	186
Supplementary Material.....	219

Summary

River communities are determined by its adaptability to the regional constrains, physical and chemical conditions, food resources and biotic interactions. Mediterranean rivers have high hydrological variations, with marked flow reduction in summer and floods in autumn and spring according to regional precipitation patterns. These hydrological changes added to the high landscape heterogeneity and the combination between arid and temperate conditions makes the Mediterranean regions a diversity hotspot. However, human water demand in some Mediterranean areas is higher than water availability producing a scenario of water scarcity and increasing human pressures as dams and pollution on rivers.

The main objective of this thesis is to *identify the effects of flow regulation and urban wastewater pollution on Mediterranean stream communities*. Although the effects of dams and urban wastewater pollution are well known at different community levels, potential effects on Mediterranean communities and, specially, on food web and species interactions have received far less attention. The present thesis will focus on: i) determine the changes that the dam's induced hydrological stability can produce on the food web structure (Chapter 1); ii) identify the effects of urban wastewater pollution on invertebrate community and functional traits under different hydrological conditions (Chapter 2); iii) analyse the

effects that wastewater pollution could produce on the energy flow along the stream food web (Chapter 3).

To meet these objectives, several field surveys were carried out on different basins tributaries of the Ebro river (NE Iberian Peninsula). To determine the changes produced by dam's induced water stability, a sampling survey was performed studying the longitudinal variation in the food-web structure in a highly-seasonal river. To identify the wastewater pollution effects on macroinvertebrate communities twelve sampling sites were sampled on summer 2015, autumn 2015 and spring 2016 up and downstream of the wastewater effluent. Ten of these sites were additionally sampled during spring 2016 to determine the pollution effects on stream food webs.

Hydrological stability caused by an irrigation dam on an intermittent stream increased the availability of autochthonous resources at the base of the food web. This, in turn, prompted a change from detritus-based to algal-based food webs downstream of the dam, increasing the richness of primary consumers. Additionally, flow stability favoured the entrance of terrestrial vegetation on the river bed as well as the predation of terrestrial invertebrates by stream top-predators. Overall, induced flow stability caused food-web structure to be longer and wider at intermediate trophic levels. However, despite a partial restitution of the flow regime, food-web structure did not recover 14 km of the dam, highlighting the overall effects of these impacts.

Pollution effects are enhanced under the lowest dilution situations produced on Mediterranean rivers; urban wastewater pollution favours the most tolerant invertebrate taxa and homogenises functional trait composition over time. Changes in functional traits were more evident during the seasonal low flow, when pollutant concentrations were at their highest downstream. However, the effects of urban wastewater pollution were not uniform, related to the local invertebrate communities variations according to the river substrata and stream size (i.e., width and discharge). Wastewater pollution reduces the energy efficiency transfer along the food web, with highest impacts on the highest trophic levels (i.e., predators). Predators show a reduction of its diet breadth, which added to a reduction of the predator-prey mass ratio and the decrease in the trophic efficiency transfer, suggest that wastewater polluted sites might be less resilient to other perturbations (e.g., floods).

The findings of this thesis highlight the importance of stream hydrology in determining ecosystem composition, stability and functioning.

Resum

Les comunitats biològiques dels rius estan determinades per la seva capacitat d'adaptació a les restriccions regionals, condicions físiques i químiques, disponibilitat de recursos i les interaccions biòtiques. Els rius mediterranis tenen una alta variabilitat hidrològica estretament lligada als patrons de precipitació, amb disminucions significatives del cabal a l'estiu i riuades a la primavera i tardor. Aquestes característiques hidrològiques juntament amb la heterogeneïtat d'hàbitats i que es troben a cavall entre els climes àrids i temperats, permeten que les regions mediterrànies presentin una gran diversitat biològica. La dependència d'aquestes regions de la precipitació, propicia que les necessitats d'aigua per a ús antròpic superin la disponibilitat, creant un escenari d'escassetat que provoca un augment de les pressions humanes en els rius com per exemple la construcció de preses i la contaminació.

Aquesta tesi té com a objectiu *identificar els efectes de la regulació del cabal i la contaminació produïda per aigües residuals urbanes en les comunitats dels rius mediterranis*. Malgrat que els efectes de la regulació i de la contaminació per aigües residuals urbanes sobre diferents comunitats biològiques han estat estudiats amb anterioritat, els efectes en rius mediterranis i, en el conjunt de la xarxa tròfica són poc coneguts. Aquesta tesi es centrarà en: i) determinar els canvis induïts per l'estabilitat hidrològica produïda per una presa sobre l'estructura tròfica (Capítol 1); ii) identificar els efectes de la

contaminació de les aigües residuals urbanes sobre la comunitat d'invertebrats i les seves característiques funcionals en diferents escenaris hidrològics (Capítol 2); iii) determinar quins efectes que pot produir aquesta contaminació sobre el flux d'energia al llarg de la xarxa tròfica.

Per assolir aquests objectius, s'ha realitzat un seguiment de diferents afluents de la part baixa del riu Ebre (NE de la Península Ibèrica). Per tal de determinar els efectes relacionats amb l'estabilitat hidrològica produïda per la presa, es va realitzar un mostreig longitudinal estudiant els canvis en l'estructura de la xarxa tròfica al llarg del riu. També es varen mostrejar dotze parells de punts, abans i després de l'abocament de les aigües residuals, per tal d'identificar els efectes originats per la contaminació de les aigües residuals urbanes a l'estiu i tardor del 2015 i la primavera del 2016. Paral·lelament, la primavera del 2016, deu d'aquests parells de punts varen ser mostrejats per determinar els efectes de la contaminació sobre les xarxes tròfiques.

L'estabilitat hidrològica produïda per una presa en un riu intermitent incrementa la disponibilitat de recursos autòctons a la base de la xarxa tròfica. Aquest fet, ha propiciat el canvi en l'ús basal dels recursos a la xarxa tròfica, des de ser basada en detritus al punt per sobre la presa a ser basades en la producció primària per sota la presa, incrementant la riquesa de consumidors primaris en aquests llocs. Per altra banda, l'estabilitat hidrològica produïda per sota la presa ha afavorit l'entrada de vegetació terrestre facilitant el consum d'invertebrats terrestres per part dels

predadors aquàtics. Aquests canvis propicien l'allargament i eixamplament de les xarxes tròfiques aigües avall de la presa. Malgrat la recuperació parcial de la variabilitat hidrològica 14 km per sota la presa, l'estructura de la xarxa tròfica no s'ha restablert, remarcant els efectes genèrics d'aquest impacte.

Els efectes produïts pels contaminants es veuen incrementats per la baixa capacitat de dilució dels rius mediterranis. La contaminació de les aigües residuals urbanes afavoreix les espècies d'invertebrats més tolerants a la contaminació, homogeneïtzant les característiques funcionals al llarg del temps. Els canvis produïts a les característiques funcionals són majors en les estacions amb cabal de riu baix, quan les concentracions de contaminants sota els efluents eren més altes. Els canvis produïts per la contaminació d'aigües residuals urbanes no varen ser uniformes, i la resposta va estar condicionada a la composició de les comunitats d'invertebrats relacionada amb la diferent composició del substrat i en la mida del riu (amplada i cabal). La contaminació per aigües residuals redueix l'eficiència en la transferència d'energia en les xarxes tròfiques, produït majors impactes als nivells tròfics superiors (predadors). Els predadors en els punts contaminats veuen reduït el seu nínxol tròfic que, juntament amb una reducció de la relació entre la massa de predador i presa i la mencionada reducció en l'eficiència de la transferència d'energia, atorguen una menor resiliència als punts impactats per aquesta contaminació, i per tant més vulnerables a d'altres perturbacions, com per exemple riudes.

Els resultats d'aquesta tesi emfatitzen la importància de la hidrologia com a factor determinant de les característiques, l'estabilitat i el funcionament dels ecosistemes.

Resumen

Las comunidades biológicas de los ríos se resultan de su adaptabilidad a los condicionantes regionales, las características físicas y químicas, la disponibilidad y el tipo de recursos, y las interacciones biológicas que se produzcan. Los ríos mediterráneos muestran una elevada variabilidad hidrológica estrechamente relacionada con la precipitación, con disminuciones significativas del caudal en verano y avenidas en primavera y otoño. Estas características hidrológicas juntamente con la alta heterogeneidad de hábitats, y el hecho que se encuentren entre climas áridos y templados, propicia que las regiones mediterráneas alberguen una gran diversidad biológica. Debido a estas características climáticas, en algunas zonas las necesidades de agua para uso antrópico superan su disponibilidad, creando un escenario de escasez que a su vez provoca un aumento de las presiones humanas en los ríos, expresada en su regulación y la presencia de contaminación.

Esta tesis tiene como objetivo *identificar los efectos de la regulación del caudal y la contaminación producida por aguas residuales urbanas en las comunidades de ríos mediterráneos*. A pesar que los efectos de la regulación y la contaminación por aguas residuales urbanas sobre diferentes comunidades han sido estudiados con anterioridad, sus efectos en ríos mediterráneos y su estudio desde una perspectiva de red trófica e interacciones entre especies han recibido una menor atención. Esta tesis se centra en: i) determinar los cambios producidos por la estabilidad hidrológica propiciada por una presa

sobre la estructura trófica (Capítulo 1); ii) identificar los efectos de la contaminación producida por aguas residuales urbanas sobre la comunidad de invertebrados y sus características funcionales en diferentes condiciones hidrológicas (Capítulo 2); iii) determinar qué efectos puede producir dicha contaminación en el flujo de energía a lo largo de la red trófica.

Para lograr estos objetivos se ha realizado un seguimiento de las comunidades en ríos afluentes de la parte baja del río Ebro (NE de la Península Ibérica). Para averiguar los cambios producidos por la estabilidad hidrológica inducida por una presa, se realizó un muestreo longitudinal con el fin de estudiar los cambios en la red trófica a lo largo del río. Para identificar los efectos originados por la contaminación debida al vertido de aguas residuales urbanas, se muestrearon doce pares de puntos, antes y después del vertido, el verano y otoño del 2015 y la primavera del 2016. La primavera del 2016 se realizó un muestreo adicional, en diez de estos pares de puntos para determinar los efectos de esta contaminación sobre las redes tróficas.

La estabilidad hidrológica producida en un río intermitente por un embalse destinado regadío, ha incrementado la disponibilidad de recursos autóctonos en la base de la red trófica. Este hecho, ha propiciado el cambio de las redes basadas en detritos, aguas arriba de la presa, a redes basadas en algas en los puntos por debajo de la presa, incrementando la riqueza de consumidores primarios en estos puntos. Paralelamente, la estabilidad hidrológica ha favorecido la entrada de vegetación terrestre, facilitando el

consumo de invertebrados terrestres por parte de los predadores acuáticos. Dichos cambios se han traducido en redes tróficas más largas y anchas en los puntos afectados por la estabilidad hidrológica. A pesar de la recuperación parcial de la variabilidad hidrológica 14 km por debajo de la presa, la estructura de la red trófica no se ha reestablecido, subrayando los efectos genéricos producidos por este impacto.

Los efectos de los contaminantes se incrementan debido a la baja capacidad de dilución de los ríos mediterráneos. La contaminación producida por las aguas residuales urbanas favorece la presencia de las especies de invertebrados tolerantes a la contaminación, homogeneizando las características funcionales de dicha comunidad de forma temporal. Los cambios funcionales se acentuaron durante los períodos en que los ríos tenían un caudal bajo, presentando los puntos por debajo del vertido las concentraciones más altas. Los cambios producidos por la contaminación de aguas residuales urbanas no fueron uniformes en todos los puntos estudiados. Cambios en la composición de las comunidades de invertebrados propiciados por diferencias en la composición del sustrato y el tamaño del río (anchura y caudal), condicionaron la respuesta de las comunidades ante la contaminación. La contaminación redujo la eficiencia en la transferencia de energía en las redes tróficas, afectando principalmente los niveles tróficos superiores (predadores). Los predadores en los puntos afectados presentan un nicho trófico más reducido que, juntamente con la reducción de la relación entre las

masas de predadores y presas, y la mencionada reducción en la eficiencia de la transferencia de energía, sugieren que los puntos afectados por esta contaminación pueden ser menos resilientes ante otras perturbaciones, tales como avenidas de agua.

Los resultados de esta tesis enfatizan la importancia de la hidrología para determinar las características, la estabilidad y el funcionamiento de los ecosistemas.

GENERAL INTRODUCTION



Rivers and who lives in them

Running waters (streams and rivers) collect water from its basin through river networks. Even though the term stream is related to smaller systems than rivers, they are usually used as synonyms and in this thesis both are used indistinctly. Rivers receive and transport water in three dimensions (Fig. I1): (I) lateral inflow, or Horton flow; (II) vertical, e.g., groundwater; and (III) longitudinal, upstream flow. These inputs are not restricted to water, since dissolved, fine and coarse matter will enter rivers through the different pathways described.

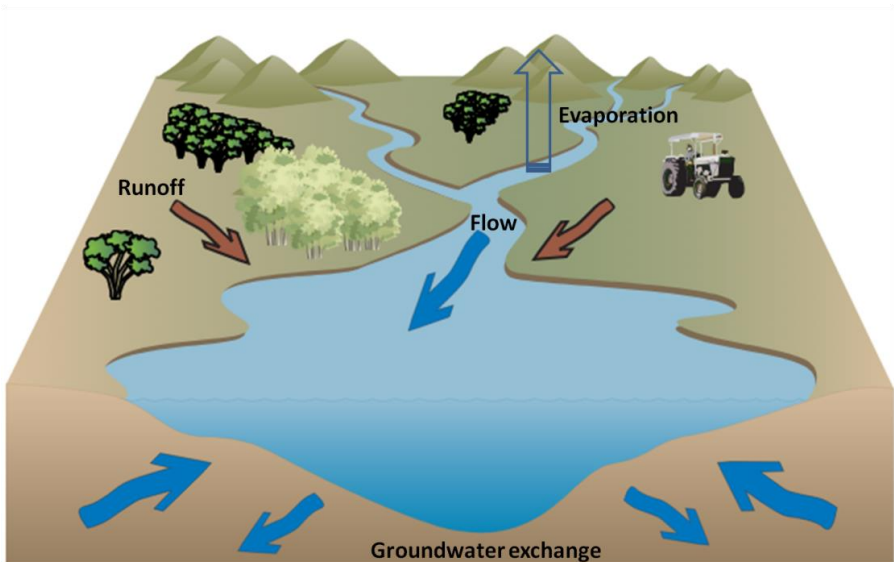


Fig. I1 River inputs and outputs of water and matter.

Rivers do not perform as inert channels. Matter and energy are transformed and used by riverine communities. Allochthonous inputs, as particulate organic matter (POM, e.g., woody debris and

leaves), dissolved organic matter (DOM) and nutrients (nitrogen and phosphorus derivatives), comes into rivers from terrestrial systems (Graça et al., 1993). The size of the particles is crucial for its processing and determines the trophic pathways. POM is leached to DOM (Allan and Castillo, 2007), colonized by fungi and bacteria and fragmented by shredders reducing the size of the particles that will be used by collectors (see below and Fig. I2). When light and adequate living conditions are suitable, primary producers (cyanobacteria, algae and macrophytes) will use dissolved inputs to grow. Autotrophic organisms are part of complex biological relationships within biofilm communities, composed of autotrophic and heterotrophic microorganisms (Wetzel, 1983).

The riverine macrofauna is composed of invertebrates, whose most diverse group are insects, and vertebrates. Both groups can have species with life cycles restricted to the aquatic environment, as invertebrates' coleopteran or fishes which larvae and adult stages lives inside the water. Other species could be a part of its life-cycle in the water and outside, as emerging insects or tadpoles with an adult stage living outside the water body. Functional traits are species characteristics related with biological (e.g., life cycle, feeding strategies), physiological (e.g., respiration technique) and ecological (e.g., microhabitat type, locomotion) attributes that let them to adapt to different habitat conditions (Tachet et al., 2010).

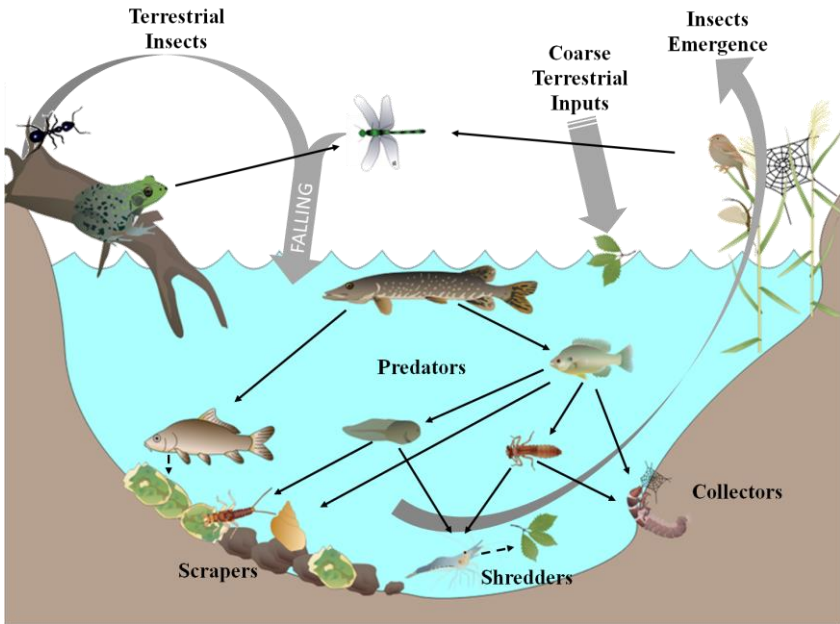


Fig. I2 River food web diagram. Food webs represent feeding interactions in a community which imply energy transfer from the sources to the top. At the base, allochthonous inputs and autochthonous production are consumed by scrapers, shredders and collectors, which will be more or less abundant according to resource quality and quantity. These groups can be predated. However, river production does not only feed freshwater organisms. Insects' emergence can be an important source for terrestrial communities as birds, spiders, bats, etc. On the same way; terrestrial insects can also fall in the water and be predated by aquatic predators.

Overall, the biological communities inhabiting river ecosystems are composed of organisms adapted to the regional constrains, physical and chemical conditions, food resources and species interactions, for example represented on the river continuum concept (Vannote et al., 1980). Stream fauna can be divided according the method used to feed on different feeding strategies that might change according the species, development stage or food availability and quality. Generally we could classify

them as: scrapers, eating biofilm and algae from stones and other surfaces (epilithic biofilm); collectors, eating fine organic matter, transported by the flow (filter-feeders) or deposited (deposit-feeders); shredders, eating and fragmenting coarse organic matter (e.g., wood debris and leaves) and consequently eating microorganisms in charge of its decomposition; and predators, eating invertebrates and/or other vertebrates (Tachet et al., 2010). Food webs account for the trophic interactions (i.e., predator-prey and competition) within the ecosystem (Post, 2002a), explaining how the energy and matter flow through it (Baird and Ulanowicz, 1989; McIntyre et al., 2007).

Studying food webs allows for the identification of basal energy inputs and that of the different pathways where energy flows up to consumers on the top of the food web. The study of food webs (see *Box 1*) has been typically used to understand how allochthonous inputs produce changes in aquatic systems (e.g., Post, 2002) and, to show that communities are far more complex networks than single interactions among species (e.g., cascading effect or key-stone species; (Wootton, 1994). It was after Nakano et al., 1999, and Power and Dietrich, 2002, when studies based on food web analysis highlighted the importance of river ecosystems for terrestrial ecosystems, for example being an important energy source for terrestrial insectivores (e.g., spiders, bats and birds).

Box 1. Food web structural metrics

Species richness: number of species.

Trophic link: feeding interaction between a consumer and a resource.

Connectance: number of realized links out of all possible trophic links.

Trophic level: number of steps energy takes from a basal resource to a determined taxa. Basal resources are considered the level 1, obligate first consumers 2, and other consumers >2.

Food chain: feeding interactions on an individual energy pathway from a basal resource to a top predator.

Omnivor: species that consume at two or more trophic levels.

Intraguild predation: when predators can consume each other.

Food-chain-length: number of trophic levels on a food chain. Usually analyzed as the maximum, shortest or mean in a food web.

Vulnerability: number of consumers using a given resource.

Generality: number of resources per consumer.

Mediterranean rivers: characteristics and anthropogenic pressures

Mediterranean regions are generally defined as semi-arid (Dallman, 1998). They are characterized by high annual and interannual rainfall seasonality. For example, the precipitation in a single location can range from ca. 200 mm/year to 1500 mm/year.

These precipitation patterns are common for all Mediterranean regions: the Mediterranean Sea basin, the California Coast (USA), the central coast of Chile, the South African Western Cape and parts of western and southern Australia. Mediterranean rivers have hydrological regimes that respond to these precipitation patterns, with high seasonal hydrological variation, a marked flow reduction in summer, and floods in autumn and spring (Gasith and Resh, 1999a). Precipitation patterns and its intensity determine drought severity which creates three hydrological typologies on Mediterranean rivers (Fig. I3; Vidal-Abarca et al., 1990): (I) permanent rivers, generally large streams or those that receive groundwater flow avoiding desiccation; (II) intermittent streams, with dry river bed periods along the year; and (III) ephemeral streams, that flow only during rainfall events.

Dry periods may represent a selective pressure for the living communities (Lytle and Poff, 2004). Mediterranean freshwater communities have evolved under constant flow variation, favouring traits that confer resistance and resilience to dry conditions. For example, biofilm communities facultatively produce mucilaginous masses and form a protective crust to avoid desiccation, and cyanobacteria synthesize protecting carotenoids (Timoner et al., 2012). Invertebrate communities exhibit biological traits such as smaller maximum size and life duration to adapt to constant flow variation, refuge-use against desiccation or higher dispersal capabilities in comparison to temperate species (Bonada et al., 2007). Lastly, strictly aquatic vertebrates (i.e., fishes) can

persist in isolated pools, being able to recolonize the river when flow conditions are restored (Lake, 2003). These adaptations to water flow variability are more frequent in Mediterranean endemic species than non-endemic species, for example in invertebrates (Tierno de Figueroa et al., 2013). The high number of endemism, the high landscape heterogeneity, the seasonal community variability according flow conditions, and the combination between arid and temperate conditions, are the main factors that contribute to Mediterranean regions being a diversity hotspot (Bonada and Resh, 2013; Dallman, 1998; Koniak and Noy-Meir, 2009; Mittermeier et al., 1999; Myers et al., 2000).

Why do we care about rivers?

In some regions, as in the Mediterranean areas, human water demand increases proportionally with the reduction of water availability (Sabater, 2008), creating a scenario of water scarcity. Under this situation, watercourses are intensively managed and modified to obtain water for human needs (i.e., agricultural, urban or industrial) by physical (e.g. water removal), pollution (e.g., chemical or by nutrients) or biological pressures (e.g., introducing invasive species). Thus, different types of pressure act on rivers by diffuse sources (e.g., agriculture pollution), or from a point source (e.g. dams or wastewater effluents).

Under this scenario of high human pressure to river ecosystems, dams and the input of urban wastewater effluents are two of the more frequent point impacts that European rivers and streams receive (EEA, 2018). Moreover, changes in water regimes

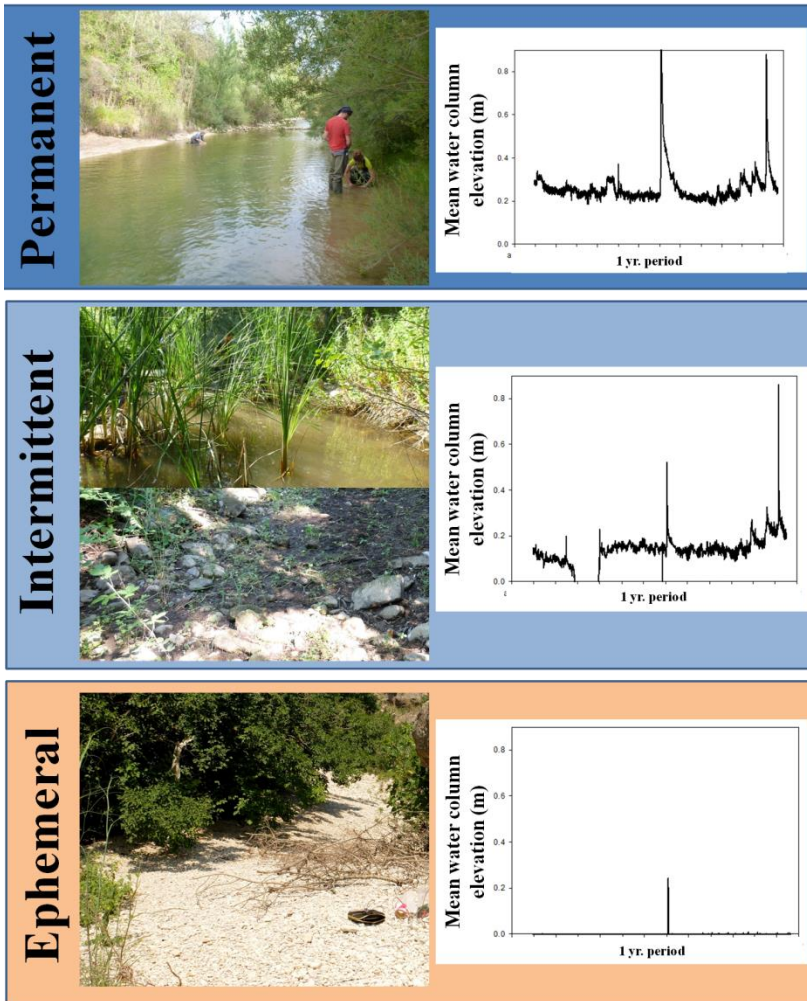


Fig. I3 Stream typologies on Mediterranean regions. Permanent (or perennial), rivers that never dry; Intermittent, these that could be dry sometimes along the year; and Ephemeral, streams on which water flows occasionally due to raining periods.

for human uses exacerbate urban wastewater pollution impacts by joining reduced dilution river capacities and increasing the pollution impact (Gasith and Resh, 1999a). Constructed dams might alter water flow regimes downstream, according to the reservoir management and purpose (e.g., hydropower, drinking

water supply, irrigation). Depending on the river hydrology (i.e., permanent or intermittent), effects might diverge. A regulated permanent river may present episodes of downstream drought when affected by a hydropower dam (Boix et al., 2010; Piqué et al., 2015). Intermittent rivers may show a reduction in intermittency and become permanent when the dam provides water for irrigation during dry periods (Batalla et al., 2004; Lobera et al., 2017). Regulated Mediterranean rivers hydrological alterations and novel conditions affect the biota inhabiting the system and its connectivity (Bonada and Resh, 2013).

Mediterranean regions can become more affected by pollution than temperate regions because of its low dilution capacity (Mandarić et al., 2018; López-Doval et al., 2013; Petrović et al., 2011). In some periods urban wastewater pollution severity can be extreme, and dominate the circulating water flow (Rice and Westerhoff, 2017). In such cases, urban wastewater inputs can be the main water input on the rivers (reaching up to 70 – 100% of the flow; Murdock et al., 2004). These effluent inputs might favour algal biomass (Carey and Migliaccio, 2009) and primary production (Aristi et al., 2015), while producing malformations of diatom cells (Tornés et al., 2018) reducing macroinvertebrate richness and diversity (Ortiz and Puig, 2007) or disturbing gonadal morphology in fish (Barber et al., 2011).

Mediterranean rivers have supported human pressures for a long time (Rundel et al., 1998). Under these conditions, freshwater communities have also evolved and have been selected

upon by the different pressures. However, new scenarios of climate change predict to reach an increase of 4°C of the freshwater water temperatures with deleterious effects on freshwater ecosystems (Dossena et al., 2012; Yvon-Durocher et al., 2011). Additionally, it is expected a decrease of the total annual precipitation and an increase of the precipitation intensity (Coumou and Rahmstorf, 2012; Trenberth, 2011). Thus, climate change will increase water scarcity around the world (Vörösmarty et al., 2000) and, consequently, the human pressure on rivers and on freshwater ecosystems in general.

Thus, although considering that dams and the urban wastewater effluents inputs are two of the more frequent point impacts that rivers receive (EEA, 2018), their effects on Mediterranean rivers have received relatively less attention (Arenas-Sánchez et al., 2016). This thesis aims to study the main effects produced by dams and urban wastewater pollution on Mediterranean stream invertebrate communities and food webs. The effects of flow regulation by dams on riverine habitat and organisms have long been studied (e.g., Brittain and Saltveit, 1989; Poff and Zimmerman, 2010; Ponsatí et al., 2015), but impacts at the higher levels of biological organization (e.g., food webs) have received relatively less attention (but see Power et al., 1996, and Cross et al., 2013). Looking the effects produced by urban wastewater pollution, previous research on Mediterranean rivers and streams are focused on biofilm community (e.g. Corcoll et al., 2015; De Castro-Català et al., 2017; Huerta et al., 2015) and

invertebrate community composition and traits (Charvet et al., 1998; Ortiz and Puig, 2007), generally analysing single cases. To achieve a wider understanding, this thesis investigate both impacts from a food web perspective and, additionally, studies the effects produced by urban wastewater pollution on invertebrates' community composition and functional traits (Box 2).

Box 2. Key methods used along this thesis

Community structure

Community structure refers to the composition of biological communities according to their abundance or biomass in different taxonomic groups or functional roles (Allan and Castillo, 2007). In this thesis community structure analyses and description were done on macroinvertebrate communities (Chapters 1, 2 and 3) and vertebrate communities (Chapters 1 and 3).

Functional traits

Life-story strategies, traits and habitat use by species are a unified way to study and measure ecosystem constrains across communities with different taxonomic composition (Statzner et al., 2001). Species traits determine ecosystem functioning reflecting actual and historical environmental conditions (Dolédec et al., 1999; Dolédec and Statzner, 2008). In this thesis functional trait analyses were developed with macroinvertebrate community (Chapter 2).

Followed on next page

Box 2 - Continued from previous page

Community Size Spectra

Body size is one of the main factors characterizing community interactions and energy flow along the food web (Brose et al., 2006; Woodward and Warren, 2006). In size-structured communities, predators generally present larger sizes than consumers. In this case, the slope between abundance and body size is proportional to the energy efficiency transfer through an ecosystem (Jennings and Blanchard, 2004). In this thesis community size spectra were done in Chapter 3. However community size analyses could also be found on Chapter 1.

Food web structure

Food web structure is basically defined as “who eats whom?” network. Food web structure is a representation of the community composition, history and organization (Post, 2002a). Food web structure provides insights into the way that energy and matter flow through ecosystems, as well as how species interact (Hairston and Hairston , 1993; Hall et al., 2000, Baird and Ulanowicz, 1989; McIntyre et al., 2007). In this thesis food web structures and its derived metrics (Box 1) were constructed by gut content analysis in Chapter 1. Food-chain length metric was also estimated using stable isotopes in Chapter 3.

Followed on next page

Box 2 - Continued from previous page

Food web niche

A 'niche' is defined as the interactions (mainly trophic) set that link the species in an ecosystem (Elton 1927). Trophic niche, understood as the result of the trophic interactions (e.g., competition, consumption/predation), has been traditionally studied with stomach content analysis (Chapter 1). However, this method is a snapshot of the trophic interactions within an ecosystem. Stable isotopes technique integrates trophic time and space interactions of the organisms (Layman et al., 2007). In this thesis the study of a food web niche using stable isotopes technique was done in Chapter 3.

AIMS



Flow regulation by dams and urban wastewater pollution are two of the more frequent point impacts on Mediterranean rivers. The main objective of this thesis is therefore to *identify the effects of flow regulation and urban wastewater pollution on Mediterranean stream communities*. More specifically, this thesis aims to answer:

- How does flow alteration driven by regulation determines stream food web structure in Mediterranean streams? Which are the impacts that flow regulation could produce on biological communities? (*Chapter 1*)
- How does taxonomical and functional diversity respond to wastewater pollution in Mediterranean streams? (*Chapter 2*)
- Is stream discharge a master variable of riverine communities structure and function? (*Chapters 1 and 2*)
- How regulation and wastewater pollution will change the energy source flowing through stream food webs? (*Chapters 1 and 3*)
- Are regulation and wastewater pollution causing the homogenization of the biological community in the river? (*Chapters 1, 2 and 3*)

These questions were approached in the thesis (Fig. A1) through the different inter-related chapters.

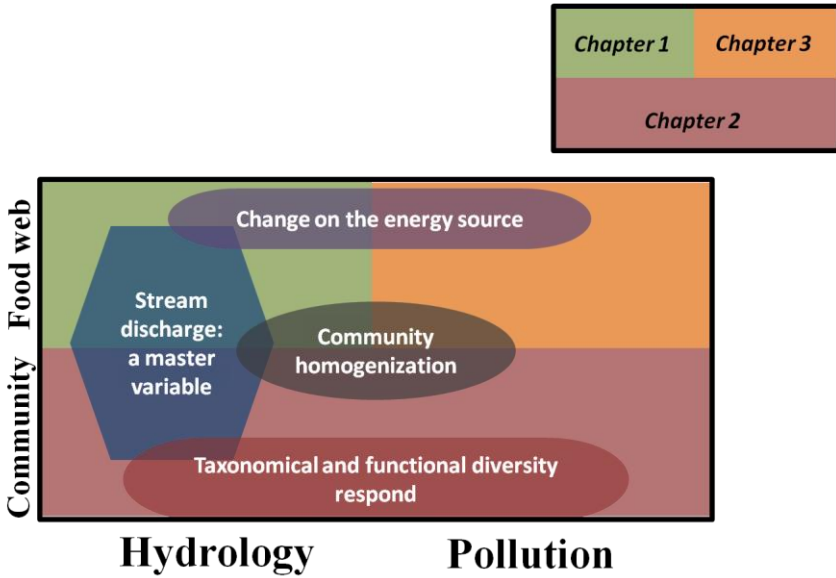


Fig. A1 Principal questions covered in the thesis, organized according to the anthropogenic impact and the community studied.

In Chapter 1, it is described the food web structure before and after a dam that produces water permanency on an intermittent river, and how much the food web structure changes along a gradient of water variability. Chapter 2 details the taxonomic and functional responses of the invertebrate community impacted by urban wastewater pollution under different hydrological situations. Finally, Chapter 3, studies the urban wastewater pollution effects on the energy flow along the riverine food web.

GENERAL METHODS



In this section the main techniques are summarized (Table M1) but specific methodological details and experimental design are provided on the Material and Method section within each chapter.

River Hydrology

- **Time series**

Hydrological time series were estimated using two different approaches. In Chapter 1, daily flow series for the 15 year series prior to the study (1998-2002) in each sampling site were obtained using stream flow time series from 1970 to 2012 using the distributed hydrological model TETIS (Francés et al., 2007), a model designed to specifically suit the hydrological cycle in Mediterranean rivers (Medici et al., 2008). In Chapter 2, water level was measured continuously (from April 2015 to May 2016, at hourly intervals) in the upstream and downstream sites, using level loggers (Solinst Levellogger, Canada), and used to derive several hydrological descriptors regarding the extension of the flow period, the tendency of a river to lose or gain water and the water level difference between the annual and a selected period mean.

		<i>Chapter 1</i>	<i>Chapter 2</i>	<i>Chapter 3</i>
River hydrology	Time series	✓	✓	
	Discharge		✓	✓
	Stream substrate	✓	✓	
Water characteristics	Physicochemical parameters	✓	✓	✓
	Inorganic nutrients	✓	✓	✓
	DOC	✓		✓
	Temperature			✓
Community and resources	Stream bed mapping	✓		✓
	Diatoms identification	✓		✓
	Biofilm Chl <i>a</i>	✓		✓
	BOM	✓		✓
	SPOM	✓		✓
	Macrophyte sampling	✓		✓
	AFDW	✓		✓
	Macroinvertebrate community composition	✓	✓	✓
	Macroinvertebrate body size and biomass	✓	✓	✓
	Macroinvertebrate functional traits		✓	
	Vertebrate community composition	✓		✓
	Vertebrate body size and biomass	✓		✓
	Gut content analysis	✓		
Stable isotopic analyses			✓	

Table M1 Summary of the methods used in each chapter of this thesis. Acronyms are explained in the text.

- **Discharge**
Instantaneous water velocity and discharge were measured by means of a snapshot cross section using an acoustic Doppler velocity meter (ADV; Flow Tracker, SonTek Handheld-AD®, P-4077).
- **Stream substrata**
Stream substrata were characterized through several transects perpendicular to the river flow. According the Wolman pebble count method (Wolman, 1954) a minimum of a 100 substratum particles per site were randomly collected, measured and categorized.

Water characteristics

- **Physicochemical parameters**
Water pH, dissolved oxygen, and electrical conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$) were measured *in situ* using hand-held probes (WTW, Weilheim, Germany).
- **Inorganic nutrients**
Phosphate ($\mu\text{g P}\cdot\text{PO}_4^{3-}\cdot\text{L}^{-1}$) and total phosphorus ($\mu\text{g P}\cdot\text{L}^{-1}$) concentrations were determined colorimetrically after Murphy and Riley (1962) using a spectrophotometer (Alliance-AMS Smartchem 140, AMS, Frepillon, France). Nitrite, nitrate and ammonium nitrogen concentrations ($\mu\text{g N}\cdot\text{NO}_3^{-}\cdot\text{L}^{-1}$; $\mu\text{g N}\cdot\text{NO}_2^{-}\cdot\text{L}^{-1}$; $\mu\text{g N}\cdot\text{NH}_4^{+}\cdot\text{L}^{-1}$) were determined using a Dionex ICS-5000 ion chromatograph (Dionex Co., Sunnyvale, USA; Hach, 2002). Total nitrogen concentrations (TN, $\mu\text{g N}\cdot\text{L}^{-1}$) were determined with a

Shimadzu TOC-V CSH coupled to a TNM-1 module (Shimadzu Co., Kyoto, Japan).

- **Dissolved Organic Carbon (DOC)**

DOC concentrations (DOC, $\mu\text{g C}\cdot\text{L}^{-1}$) were determined with a Shimadzu TOC-V CSH coupled to a TNM-1 module (Shimadzu Co., Kyoto, Japan). The water samples were previously filtered in 0.7 μm GF/F filters (Whatman Int. Ltd., Maidstone, UK) and kept at $-20\text{ }^{\circ}\text{C}$ until analyzed.

- **Temperature**

Further to instant measurements, continuous water temperature ($^{\circ}\text{C}$) were recorded hourly with a data-logger (Solinst Levelogger, Canada).

Community and resources

- **Stream bed mapping**

Basal resources available to primary consumers (coarse benthic organic matter (CBOM), fine benthic organic matter (FBOM), biofilm patches and macrophytes) were identified *in situ* using an underwater viewer (30 x 30 cm). The relative cover of these basal resources was recorded every 30 cm along ten cross-sectional transects at each reach. Roots and organic matter were also included in the mapping. Identification and mapping included the different patches of epilithic biofilms, macrophytes and bryophytes, and detritic organic matter. Biofilm patches were separated by their colour, mucilage, macrocolonies of cyanobacteria, and macroalgae presence.

- **Diatom community identification**
Diatom cells were cleaned in boiling hydrogen peroxide, and cleaned frustules were mounted on permanent slides using Naphrax (r.i. 1.74; Brunel Microscopes Ltd., Chippenham, Wiltshire, UK). Up to 400 valves were counted on each slide by performing random transects under light microscopy (Nikon Eclipse 80i; Nikon, Tokyo, Japan) using Nomarski differential interference contrast optics at a magnification of 1000x. The non-diatom algal fraction was determined after counting 50 random microscope fields per aliquot (Tornés and Sabater, 2010).
- **Biofilm chlorophyll-a (Chl-a)**
Biofilm samples were lyophilized and Chl-*a* was extracted with 90% v/v acetone (-4 °C, 12h), extracted with acetone 90% and estimated after Jeffrey and Humphrey (1975).
- **Benthic organic matter (BOM)**
Coarse benthic organic matter (CBOM) and fine benthic organic matter (FBOM) were collected using a sediment corer (314 cm³). Samples were frozen (-20 °C) and processed to obtain ash free dry weight (AFDW, in g·m⁻²).
- **Suspended organic matter (SPOM)**
SPOM samples were obtained by filtering stream water through a precombusted (450°C, 4h) Whatman GF/F filter using a manual vacuum pump. Samples were frozen (-20 °C) and processed to obtain ash free dry weight (AFDW, in g·m⁻²).

- **Macrophyte sampling**
Macrophytes were identified at species level and collected from a known area (30 x 30 cm) according to the streambed mapping. Samples were conserved on a fridge, cleaned from attached material (e.g., algae or debris) and processed to obtain ash free dry weight (AFDW, in $\text{g}\cdot\text{m}^{-2}$).
- **Ash-free dry weight (AFDW)**
Samples (biofilm, BOM, POM and macrophytes) were dried (60 °C, to constant weight), weighed, combusted (450 °C, 4h), and reweighed to consider the ashes weight (AFDW).
- **Macroinvertebrate community composition**
Macroinvertebrate communities were sampled using a Surber net (30 x 30 cm, mesh size 250 μm) or a core sampler ($\text{Ø} = 25$ cm) according to stream substratum (Rodrigues-Capítulo et al., 2009). The samples were preserved in 4% formalin, and invertebrates were sorted, counted and identified at the lowest taxonomic level possible, mostly to the level of genus. Density was calculated referred to sampler surface.
- **Macroinvertebrates body size and biomass**
The first twenty-five individuals of each taxon at each sample were measured using an ocular reticule (± 0.1 mm), from which biomass (mg dry weight) were derived using published length-mass relationships (Benke et al., 1999; Burgherr and Meyer, 1997; Meyer, 1989; Ramsay et al., 1997; Sabo et al., 2002).

- **Macroinvertebrates functional traits**

The frequency of each trait category by species was distributed considering the species variability by the fuzzy code method (Statzner and Bêche, 2010) using public databases for European macroinvertebrate taxa (mainly at the genus level; Tachet et al., 2010), with some adaptations for the Mediterranean region (Bonada et al., 2007; Bonada and Dolédec, 2011). We then multiplied the frequency of each trait category by the relative log-transformed abundances of taxa in the sites to obtain the relative utilization of each trait category in each site.
- **Vertebrates community composition**

Aquatic vertebrates (fish, amphibians and reptiles) were characterized by three runs in an enclosed reach by electrofishing technique (SmithRoot backpack electrofisher; 200–350 V, 2–3 A fully rectified triphasic DC).
- **Vertebrate body size and biomass**

All vertebrate individuals were measured and weighted in the field.
- **Gut content analyses**

Gut content analyses was done by ocular identification. Animal material was identified at the lowest taxonomic level possible, mostly genus, using a dissecting microscope at 80x magnification. Non-animal material was stained with Rose Bengal and classified as detritus, bacteria, vegetal

material, fungi, non-filamentous algae, filamentous algae, or diatom, using a phase-contrast microscope (600 x).

- **Stable isotopes analysis (SIA)**

Samples for SIA were classified, cleaned, dried, homogenized, weighted and capsulated. Stable isotopes of C and N were analyzed on a Flash 1112 elemental analyzer connected to a Delta C isotopic ratio mass spectrometer with ConFlo III interface (Thermo Scientific, Inc.) at the Scientific and Technological Centers- University of Barcelona.

Study sites

Research for this thesis was carried out in ten different catchment tributaries of the Ebro River. The Ebro River, located in the north-east of the Iberian Peninsula, covers a surface of 85362 Km² from the Cantabrian sea to its delta on the Mediterranean coast (Fig. M1a). The Ebro basin counts 260 dams higher than 10 m, 187 of those in the main river course. However, in the basin, the number of smaller dams and weirs reaches 4000 (CHE, 2013). Main land use is agricultural (49.7%) and forested (28.5%) having low population densities. The Ebro basin counts with 1783 urban wastewater inputs of towns with more than 250 inhabitants, being the 54.3% of those non-treated (CHE, 2013). The studied streams were located in the lower Ebro catchment (Fig. M1b), all having a Mediterranean climate characterised by dry summer periods with marked flow reductions and rainfall episodes in spring and late autumn (Sabater et al., 2009).

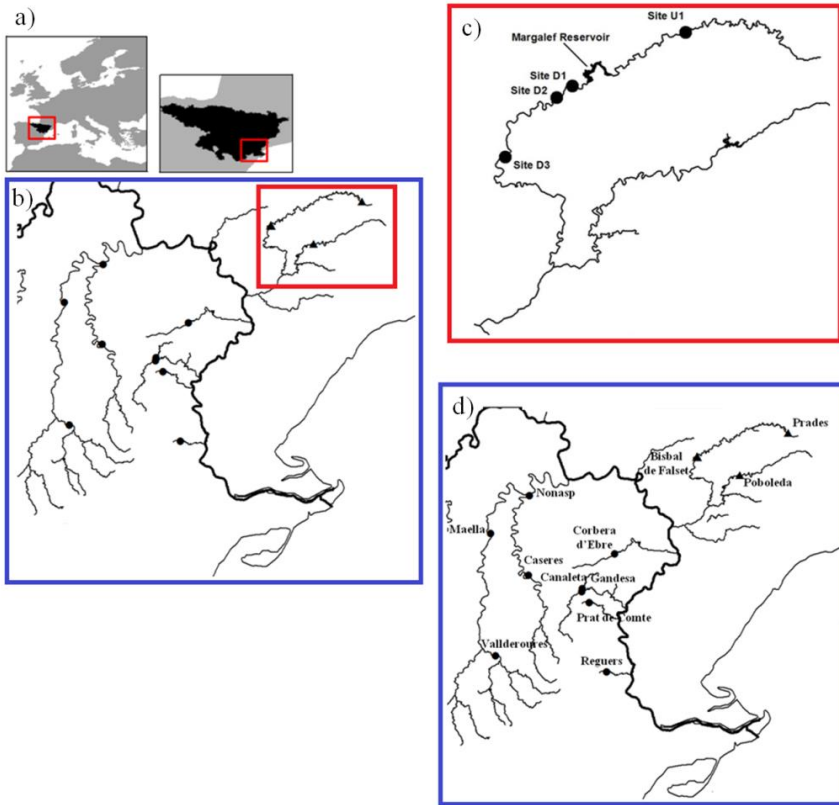


Fig. M1 Study sites map. Study sites were located in ten rivers of the Ebro basin (a and b). For Chapter 1 for sites, one upstream and three downstream of the Margalef reservoir were studied (c). For Chapters 2 and 3, twelve and ten sites were selected in ten different rivers (d). See main text for details.

In Chapter 1, we selected four sites in the Siurana basin. One site located upstream and three downstream of the Margalef reservoir (built in 1995 for irrigation; 3 hm^3 nominal volume, 33.2 m dam height, 0.3 years residence time) on the Montsant River (Fig. M1c). The Montsant River is a naturally intermittent river (Mate et al., 2013), considered as near-pristine by the riverine-riparian bioassessment index ECOSTRIMED (Bonada et al., 2006). The Montsant catchment covers a surface of 618 Km^2 mainly covered

Stream site	Basin	Chapter	Effluent	Resident Population	Land use (%)					
					UR	AG	FO	GR	RI	RE
Prades	Prades	2 & 3	WWTP	587	4.7	43.0	48.7	1.3	2.4	0
Bisbal de Falset	Montsant	2 & 3	WWTP	208	0.6	22.6	44.8	27.8	4.1	0
Poboleda	Siurana	2 & 3	WWTP	374	0.4	23.6	50.3	20.8	4.3	0.6
Nonasp	Algars	2 & 3	Untreated	992	0.1	49.9	33.4	12.3	4.3	0
Caseres	Algars	2	Untreated	253	<0.1	45.2	39.9	10.7	4.2	0
Maella	Matarranya	2 & 3	Untreated	1970	0.1	35.8	44.7	15.0	4.3	0.1
Vallderoures	Matarranya	2 & 3	Untreated	2311	0.2	12.0	61.6	21.5	4.3	0.4
Canaletes	Canaletes	2 & 3	Untreated	615	<0.1	28.9	52.9	14.0	4.2	0
Gandesa	Gandesa	2 & 3	Untreated	615	<0.1	67.6	27.3	1.1	4.0	0
Prat de Comte	Xalamera	2 & 3	Untreated	177	<0.1	33.7	47.5	14.3	4.5	0
Reguers	Cervera	2	Untreated	653	0.9	24.7	37.2	32.8	4.5	0
Corbera d'Ebre	Sec	2 & 3	Untreated	1080	1.5	84.8	9.2	0.4	4.3	0

Table M2 Characteristics of the studied sites of Chapters 2 and 3. Effluent types (tertiary wastewater treatment plants (WWTP) and untreated effluents), resident population (2016) and % land use cover upstream at the studied sites (UR = Urbanized, AG = Agricultural, FO = Forested, GR = Grasslands, RI = river, RE = reservoir).

by forest (45% of the catchment land use), agriculture (26%) and grasslands (26%) having a low population density (<1% of the land use).

Chapters 2 and 3 were conducted on sites located before and after urban wastewater effluents inputs. We therefore used 12 reaches from 10 catchments (Fig M1d; Table M2). All the studied locations were situated in low-mountain Mediterranean areas, ranging from 365 m to 950 m of altitude. The basins were mainly forested, with low agriculture intensity and pasture activities, and low population density (Table M2). The downstream sites had a complete mixture of effluents and stream waters (identified by an homogenous conductivity across a stream section during the rainy season). The upstream and downstream sites were situated between 200 m and 1300 m from each other, depending on the stream size. Differences in land use were minimal between up and downstream sites and no tributaries or water flow diversions intervened between them. Overall, the only difference between up and downstream sites was the discharge of urban wastewater effluents into the downstream sites. Nine pairs of sites received untreated urban effluents whereas the remaining three received treated effluents from tertiary wastewater treatment plants (Table M2).

Thesis Chapters

This section contains three papers that constitute the main body of the thesis. Supplementary material is attached at the end of the thesis and larger tables and figures could be found on the join CD, on online published papers or contacting with the author, so as not to waste paper.

CHAPTER 1

Dam regulation and riverine food-web structure in a Mediterranean river

Mor, J.R., A. Ruhí, E. Tornés, H. Valcárcel, I. Muñoz and S. Sabater. SCIENCE OF THE TOTAL ENVIRONMENT. 2017. **625**: 301-310.

Background

Discharge might be considered a ‘master variable’ that structures riverine habitat, influences water quality, and controls population and community dynamics and many ecosystem processes (Death and Winterbourn, 1995; Bunn and Arthington, 2002). Flow regimes control channel morphology and size, habitat diversity (riffles and pools) and substrate stability, which together influence the abundance, distribution, and diversity of organisms (Power et al., 1995; Nilsson and Svedmark, 2002). Flow variation is positively associated with allochthonous inputs of matter and energy (Tank et al., 2010), with the amount and seasonality of organic matter transport and accumulation (Uehlinger, 2000; Artigas et al., 2009), and with hydrologic connectivity (Jaeger et al., 2014; Ruhí et al., 2015b). Because riverine communities are adapted to natural flow variability, flow alteration poses a major risk for the stability and functioning of aquatic ecosystems, changing both abiotic and biotic parameters (Poff and Zimmerman, 2010; Carlisle et al., 2011).

Dams occur worldwide (Nilsson et al., 2005) regulating most of the discharge in the northern hemisphere (Dynesius and Nilsson, 1994) and threatening some of the world’s most biodiverse rivers (Winemiller et al., 2016). When intermittent rivers become regulated for human supply and irrigation, novel conditions for this adapted biota are created, increasing hydrological stability via dampened flood frequency and drought severity (Batalla et al., 2004; Döll et al., 2009). This flow stability

can in turn change the flux of materials and energy (Abril et al., 2015); can act as a dissolved nitrogen (N) sink, causing relevant N cycling discontinuities (von Schiller et al., 2016); can enhance organic carbon processing (Aristi et al., 2014); and can favour biofilm biomass growth, reducing biofilm spatial heterogeneity and habitat quality (Belmar et al., 2013; Ponsatí et al., 2015). Altogether, these effects can ripple through the food web (Power et al., 2013) and result in altered trophic links, energy pathways, and food-web dynamics (Vander Zanden et al., 1999).

The study of food-web structure provides insights into how energy and matter flow through ecosystems (Baird and Ulanowicz, 1989; McIntyre et al., 2007). Food webs arise from community composition and interactions among taxa (Post, 2002a); thus, food-web structure is sensitive to changes in biodiversity (in the form of local extinction and colonization) as well as to changes in the sign or strength of interactions that exist among organisms (Post and Takimoto, 2007). However, riverine food-web structure also integrates exogenous disturbance, community history and resource availability and type (i.e., allochthonous *vs.* autochthonous) (Post, 2002). Thus, understanding how food-web structure responds to anthropogenic disturbance can shed light into designing effective conservation strategies (McCann, 2007; Harvey et al., 2017).

Here we investigated to what extent food-web structure in an intermittent Mediterranean stream was affected by the presence of an irrigation dam that altered the river ecosystem by reducing

the downstream flow variability. We also aimed to see whether food-web structure recovered downstream, in parallel with the progressive recovery of hydrological conditions due to the inputs of intermittent tributaries. To address these questions, we selected sites differing in flow regime but sharing the regional pool of species, and we studied local food webs via gut content analyses and food-web structure metrics. We predicted that: (1) Dam-induced hydrological stability should increase the availability of autochthonous resources downstream of the dam (Ponsatí et al., 2015). Because autochthonous resources generally have lower C:N and C:P ratios (Frost et al., 2002), and higher protein and lipid content than terrestrial matter (Lamberti, 1996), autochthony should favour herbivory over detritivory. (2) This shift should increase primary consumer diversity, increasing food-web width (via a higher diversity of trophic pathways) and food-chain length (FCL). This result would be in agreement with the dynamic stability or ‘disturbance’ hypothesis of FCL (Pimm and Lawton, 1977; John L Sabo et al., 2010). (3) Finally, we hypothesized that these effects should be reduced downstream, as flow variability is often progressively recovered with increasing distance from the dam (Batalla et al., 2004). Research on this topic may help anticipating the effects of increasing flow regulation by dams on riverine food webs, a critical question given the steep increase in dam building across the globe (Zarfl et al., 2016).

Material and Methods

Study location

A survey was conducted during May 2012 in the Montsant River, an intermittently-flowing tributary of the Ebro River (NE Iberian Peninsula). Basal resources, invertebrate, and vertebrate samples were collected from four river segments (Fig. 1.1). These were an upstream site (U1) located 12.3 km upstream of the Margalef reservoir, and three sites downstream of the dam: D1 (1.3 km downstream of the dam), D2 (3.4 km downstream of the dam), and D3 (14.2 km downstream of the dam). Sampling was carried out in spring, as Mediterranean-climate river communities peak in species richness during that season (Gasith and Resh, 1999a). This way, riverine food webs could be depicted in their full complexity. The sampling reaches were 100-m long, all including one pool (central part of the site) and two riffles (upstream and downstream of the pool). The Montsant River is classified as a mineralized, low-mountain Mediterranean river (R-M2) by the EU Water Framework Directive 2000/60/EC (European Union Council, 2000), and is considered a near-pristine by the riverine-riparian bioassessment index ECOSTRIMED (Núria Bonada et al., 2006). The Montsant River is naturally intermittent, with dry periods during summer and sudden floods during spring and autumn (Mate et al., 2013). The Margalef reservoir (built in 1995 for irrigation; 3 hm³ nominal volume, 33.2 m dam height, 0.3 years residence time) laminates floods and provides permanently-flowing conditions, reducing downstream flow variability.

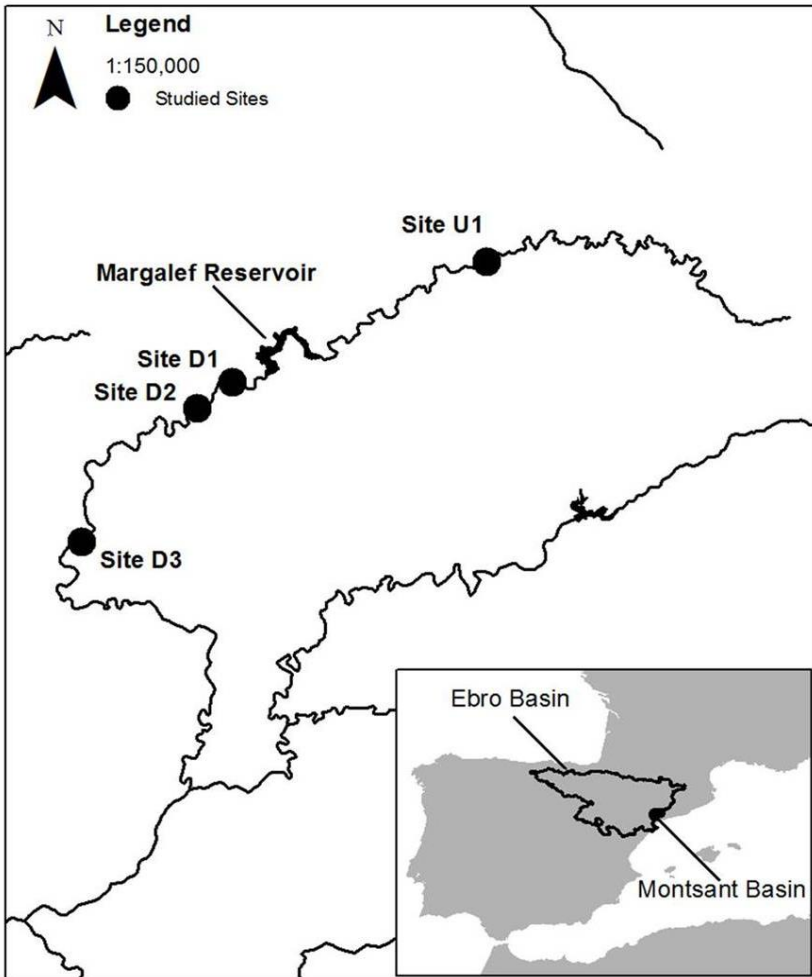


Fig. 1.1 Locations of the study sites in the Montsant River, upstream (U1), and downstream (D1, D2, D3) of Margalef reservoir.

Hydrological characterization

Streamflow time series (1970-2012) at each site were obtained using the distributed hydrological model TETIS (Francés et al., 2007), a model designed to specifically suit the hydrological cycle in Mediterranean rivers (Medici et al., 2008). The presence of

reservoirs was included in the model, together with topographical, geological, soil, and land use information, considering the presence of the reservoir and bypasses. The model was calibrated and validated in the watershed with a dataset of 13 years of daily streamflow at the inflow of the Siurana Reservoir (reservoir at the same basin, Nash-Sutcliffe efficiency = 0.67; see Ruhí et al., 2016, for details), and delivered daily flow series for the 15 years prior to the study (1998-2012). We analyzed these series with the Discrete Fast Fourier Transform [DFFT; (Sabo and Post, 2008)], and we quantified flow variability by adding up the number of daily high- and low-flow events over the 15 year series, with events being defined as flows falling beyond the $1 \pm$ standard deviation threshold in the distribution of residuals or ‘anomalies’ (Sabo and Post, 2008).

Environmental characterization

Electrical conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$), pH, and water temperature ($^{\circ}\text{C}$) were measured in situ using hand-held probes, three times during the sampling day (WTW, Weilheim, Germany). Three water samples in each reach were collected for nutrient analyses (nitrate (NO_3^- , $\mu\text{g}\cdot\text{L}^{-1}$), nitrite (NO_2^- , $\mu\text{g}\cdot\text{L}^{-1}$), ammonium (NH_4^+ , $\mu\text{g}\cdot\text{L}^{-1}$), phosphate (PO_4^{3-} , $\mu\text{g}\cdot\text{L}^{-1}$), and for dissolved organic carbon analyses (DOC, $\mu\text{g}\cdot\text{L}^{-1}$). The water samples were filtered in $0.7\ \mu\text{m}$ GF/F filters (Whatman Int. Ltd., Maidstone, UK) and kept at $-20\ ^{\circ}\text{C}$ until analyzed. Phosphate concentration was determined colorimetrically using a spectrophotometer (Alliance-AMS Smartchem 140, AMS,

Frepillon, France), after Murphy & Riley, (Murphy and Riley, 1962). Nitrite, nitrate, and ammonium concentrations were determined on a Dionex ICS-5000 ion chromatograph (Dionex Co., Sunnyvale, USA; Hach, 2002). DOC concentration was determined on a Shimadzu TOC-V CSH coupled to a TNM-1 module (Shimadzu Co., Kyoto, Japan).

Streambed mapping

Basal resources available to primary consumers (coarse benthic organic matter (CBOM), fine benthic organic matter (FBOM), biofilm patches and macrophytes) were identified *in situ* using an underwater viewer (30 x 30 cm). The relative cover of these basal resources was recorded every 30 cm along ten cross-sectional transects at each reach. Roots and organic matter were also included in the mapping. Identification and mapping included the different patches of epilithic biofilms, macrophytes and bryophytes, and detritic organic matter. Biofilms were separated by their colour, mucilage, macrocolonies of cyanobacteria, and macroalgae presence.

Biofilm collection and identification

Five stones for each of the three most representative biofilm patches (i.e., % cover) at each reach were randomly collected. Two subsamples were taken for the taxonomic analysis, one for diatoms and another for the non-diatom algae and cyanobacteria. Algae were scraped off to a final area of 2-10 cm², and preserved in 4% formalin until analysis. Five replicates of 2-10

cm² were taken for chlorophyll analysis, stored in the dark and frozen in the field (-20 °C) until analysis.

Diatom cells were cleaned in boiling hydrogen peroxide, and cleaned frustules were mounted on permanent slides using Naphrax (r.i. 1.74; Brunel Microscopes Ltd., Chippenham, Wiltshire, UK). Up to 400 valves were counted on each slide by performing random transects under light microscopy (Nikon Eclipse 80i; Nikon, Tokyo, Japan) using Nomarski differential interference contrast optics at a magnification of 1000x. The non-diatom algal fraction was determined after counting 50 random microscope fields per aliquot (Tornés and Sabater, 2010). We also collected coarse benthic organic matter (CBOM) and fine benthic organic matter (FBOM) using a sediment corer (314 cm², 3 replicates/reach). Samples were frozen (-20 °C) and processed to obtain ash free dry weight (AFDW, in g·m⁻²). Values were transformed to carbon weight after Margalef (1986).

Macrophyte and bryophyte collection and identification

For species identification we collected macrophytes and bryophytes when present. For biomass analysis, an area of 90 cm² was collected for each species, and samples were preserved in zip-lock bags at 4 °C in the field until analysis.

Macroinvertebrate and vertebrate sampling and gut content analysis

At each sampling site, ten Surber sample-units (30 × 30 cm square; mesh aperture 500 µm) were collected, integrating the different microhabitats present in riffle (six samples) and pool habitats (four samples). These benthic samples were preserved in 4% formaline.

Vertebrates (fishes and frogs) were captured via electrofishing, using a 3-pass depletion method along the same section of the river (100 m), after closing it with blocking nets. All vertebrate individuals were measured and weighted, and up to twenty individuals of each fish species and size class were euthanized with an overdose of anaesthetic (MS-222) and frozen for gut content analysis (protected or vulnerable species were not euthanized).

Gut content analysis was used to determine feeding links among species. Twenty individuals of each taxon, developmental stage (e.g., larval instar, pupa or adult) and size class (in the case of vertebrates) were randomly selected at each site, stomach tracts were extracted, and gut contents were carefully removed and classified as animal or non-animal material under a dissecting microscope at 80x magnification. Invertebrate non-animal contents were measured in volume and transformed to dry weight using derived volume-mass ratio transformations (see Appendix S1.1), and vertebrate non-animal material were dried at 60 °C during 24 h and weighed (g dry mass). Given the low biomass of

non-animal material in invertebrate gut contents, groups of four invertebrate stomachs of the same taxon, size and site were pooled together for subsequent non-animal analysis; meanwhile, each vertebrate stomach was a sample. Non-animal material was stained with Rose Bengal and classified as detritus, bacteria, vegetal material, fungi, non-filamentous algae, filamentous algae, or diatom, using a phase-contrast microscope (600 x). For each sample, fifty random fields were counted in order to assign categorical abundance values to each group. Animal material was identified to the lowest taxonomic level possible. Individuals were counted, and the first twenty-five individuals of each taxa were measured using an ocular reticule (± 0.1 mm). All chironomids were identified to genus level. Biomass (mg dry weight) was calculated using published length-mass relationships (e.g., Burgherr and Meyer, 1997; Benke et al., 1999).

Network structure properties

Food-web structure properties at each site were calculated using the Network3D (Yoon et al., 2004; Williams, 2010) and the Cheddar (Hudson et al., 2013) software. Network3D provided species, link, and omnivory properties analysis, and Cheddar provided the fraction of links between trophic levels, food chain properties, and consumer-prey asymmetries. These network metrics included: species richness (number of species present in the food-web), fraction of top species (number of species not preyed upon), intermediate species (consumer species preyed upon) and basal species, link density (number of links per species),

connectance (number of realized links out of all possible trophic links), mean food-chain length and maximum food-chain length, omnivory (species that consume at two or more trophic levels), vulnerability (number of consumers using a given resource), generality (number of resources per consumer), and associated normalized standard deviations. For taxa with no stomach content, links were established using the available literature (see Table S1.1). Best link options and strengths were informed based on trophic position estimates obtained via C and N stable isotope analyses in the same catchment (Ruhí et al., 2016).

Data analysis

A Permutational Multivariate ANOVA (PERMANOVA) was used to test for differences in basal resource structure (biomass) across sites. Because 10 cross-sectional transects were made per site, ‘transect’ was treated as a random factor and nested within ‘site’ (fixed factor). In order to detect differences on a given variable across sites, we employed a Permutational Analysis of Variance (PERANOVA) following the same design as for the PERMANOVA, and pair-wise PERMANOVA tests were used to compare pairs of sites. In all cases, Euclidian distances were computed on fourth-root transformed data. We ran PERMANOVA and PERANOVA, using 999 permutations on PRIMER-E 6 v.6.1.11 and PERMANOVA+ v.1.0.1 (PRIMER-E Ltd., Plymouth, UK).

Results

Hydromorphological characterization

Mean annual streamflow did not differ significantly among the studied sites (PERANOVA, pseudo- $F_{3,891}=2.5817$, $p=0.075$). However, flow variability was higher upstream (site U1) than downstream of the dam. The lowest variability was observed at site D1, where the number of low and high flow events was drastically reduced (relative to the upstream site). Flow variability progressively recovered in the downstream sites D2 and D3 (Table 1.1). The streambed substrata consisted of pebbles ($> 80\%$) and cobbles at all sites, with sediment diameter size decreasing downstream (Table 1.1). Stream width and depth also changed longitudinally, with channel width being reduced immediately downstream of the dam.

Basal resource characterization

Dominant basal resources differed across sites (PERANOVA, Macrophyte: pseudo- $F_{3,555}=19.051$, $p=0.001$; Algae: pseudo- $F_{3,555}=18.151$, $p=0.001$), with macrophytes dominating at site U1 and different algal patches dominating at all other sites (Table 1.2). Denuded tree roots substrata were restricted to impact sites (Table 1.2). Main basal resources were organic matter (CBOM and FBOM), diatoms (see Table S1.2), cyanobacteria and non-diatom algae (filamentous and non-filamentous algae, see Table S1.3), bryophytes (*Fontinalis antipyretica* and *Rhynchostegium riparioides*), and macrophytes (*Groenlandia densa*, *Lemna minor*, *Mentha aquatica*, *Potamogeton coloratus*, *Ranunculus aquatilis*, *Ranunculus repens*, and

Environmental variables	Site U1	Site D1	Site D2	Site D3
Basin area (km ²)	40.7	97.6	113.1	141.4
Basin regulation area (%)	0	95	82	65.5
Observed intermittence	Yes	No	No	No
Number of low flows	72	30	64	73
Number of high flows	70	28	47	70
Channel width (m)	6.0 ± 3.1	4.0 ± 1.4	3.4 ± 1.0	5.1 ± 2.3
Mean rock diameter (mm)	50.6 ± 3.5	45.5 ± 2.6	40.3 ± 2.0	31.5 ± 1.6
Pebbles substratum (%)	82.1	80	93.4	98
Cobbles substratum (%)	16.1	20	6.6	2
Light	Exposed	Shaded	Shaded	Exposed
T (°C)	11.9	13.7	13.4	13.1
Conductivity (µS·cm ⁻¹)	365.7 ± 0.6	412.0 ± 3.0	432.0 ± 0.0	485.0 ± 0.0
DO (mg O ₂ ·L ⁻¹)	9.6 ± 0.4	12.1 ± 0.0	10.6 ± 0.0	7.8 ± 0.0
DOC (mg C·L ⁻¹)	1.8 ± 0.1	3.0 ± 0.1	2.4 ± 0.3	1.7 ± 0.2
PO ₄ ³⁻ (µg P·L ⁻¹)	12.6 ± 0.7	8.0 ± 0.0	8.0 ± 0.0	5.2 ± 1.4
NO ₂ ⁻ (µg N·L ⁻¹)	3.0 ± 5.3	5.8 ± 0.5	5.9 ± 1.6	5.0 ± 3.5
NO ₃ ⁻ (µg N·L ⁻¹)	2.5 ± 0.8	533.4 ± 4.1	377.2 ± 4.5	27.5 ± 5.7
NH ₄ ⁺ (µg N·L ⁻¹)	4.5 ± 6.1	1.4 ± 0.7	0.9 ± 0.5	0.9 ± 0.3
TDN (µg N·L ⁻¹)	157.5 ± 7.7	686.1 ± 18.4	442 ± 83.9	177.5 ± 23.5

Table 1.1 Hydromorphological and water variables at each study site. The number of low and high flow days integrates the 15 years prior to the study and were obtained with DFPT analysis of TETIS model outputs.

Substrate	Site U1	Site D1	Site D2	Site D3
Root	-	-	4.7 ± 16.7	3.4 ± 14.4
Algae	26.5 ± 34.9	83.9 ± 32.2	75.9 ± 32.1	60.1 ± 42.7
Bryophyte	2.6 ± 11.4	8.7 ± 21.6	9.9 ± 17.1	20.3 ± 30.6
Macrophyte	70.9 ± 38.0	6.4 ± 19.9	9.5 ± 19.8	16.2 ± 33.4

Table 1.2 Stream relative cover proportion (%) at each site.

Rorippa nasturtium-aquaticum). Macrophytes and CBOM were the greatest contributors to total basal resource biomass at site U1 (Table 1.3). Macrophyte biomass at site U1 was significantly higher than at the other sites (PERANOVA Pair-wise test, $p < 0.005$). Bryophyte biomass slightly increased downstream, but there were not significant differences among sites (PERANOVA, pseudo- $F_{3,555} = 1.654$, $p = 0.169$). Algal biomass increased at site D1, presenting no difference with D2 but significantly decreased downstream (site D3, PERANOVA Pair-wise test, $p < 0.01$). Benthic organic matter (CBOM and FBOM) did not differ among sites (PERANOVA, CBOM: pseudo- $F_{3,8} = 1.195$, $p = 0.37$; FBOM: pseudo- $F_{3,8} = 3.515$, $p = 0.078$).

Basal resource	Site U1	Site D1	Site D2	Site D3
CBOM (g m ⁻²)	67.9 ± 92.7	72.6 ± 20.6	132.5 ± 28.0	76.3 ± 37.1
FBOM (g m ⁻²)	14.3 ± 12.0	106.8 ± 82.9	180.2 ± 129.2	62.8 ± 69.0
Algae (g m ⁻²)	0.2 ± 0.2	1.5 ± 0.9	1.2 ± 0.7	0.8 ± 0.6
Bryophyte (g m ⁻²)	2.9 ± 12.7	4.9 ± 13.0	6.4 ± 12.1	6.6 ± 11.3
Macrophyte (g m ⁻²)	64.3 ± 34.7	1.0 ± 3.5	1.5 ± 3.5	6.7 ± 18.8

Table 1.3 Coarse benthic organic matter (CBOM), fine benthic organic matter (FBOM), algae, bryophyte, and macrophyte biomass at each site (mean ± SD).

Consumer characterization

A total of eight vertebrate taxa (*Pelophylax perezii*, *Natrix maura*, *Parachondrostoma miegii*, *Barbus haasi*, *Luciobarbus graellsii*, *Gobio lozanoi*, *Anguilla anguilla*, and *Salmo trutta*) and 62 invertebrate taxa (see Table S1.4) were observed in the study sites. Invertebrate species richness was similar among sites, whereas community diversity decreased with dam impacts and recovered downstream (Table 1.4). Invertebrate composition differed between upstream and downstream sites. The upstream site presented 9 exclusive taxa - the most abundant being *Nemoura* sp. - and shared 20 taxa with the downstream sites (including the highly-abundant *Ancyclus fluviatilis* and Orthocladiinae species). Downstream sites showed 41 taxa absent from site U1, with *Caenis* sp. and *Ephemera* sp. being the most abundant ones. Sites D1 and D2 shared 20 taxa (see Table S1.4). Orthocladiinae was the most abundant invertebrate group in all sites. When this group was not considered in the analyses, invertebrate abundance decreased by the dam (site D1) and recovered downstream (Table 1.4).

Fish were not present in the upstream site, where the water snake *Natrix maura* and the frog *Pelophylax perezii* were the only aquatic vertebrates present. Fish occurred at all downstream sites, with most fish species being common at all three sites except *Anguilla anguilla*, which was only present in D1 and D2. Vertebrate densities were relatively high at impact sites (Table 1.4).

Consumer diet description

Macroinvertebrate diet was composed (in decreasing biomass) of invertebrates, diatoms, detritus, vegetal material, dead animal material, filamentous algae, fungi, and non-filamentous algae (Table S1.1). Vertebrates were largely herbivorous at all sites, although insectivore taxa were also abundant at sites D1 and D2. Vertebrate diets included terrestrial invertebrates at site U1, where *P. perezii* based 75.3% of its whole diet weight on *Gryllotalpa gryllotalpa* and Formicidae; and at site D2, where *G. lozanoii*, *B. haasi* and *L. graellsii* based 84.6%, 46.2% and 0.3% of their respective diet weights on Formicidae, other Hymenoptera, terrestrial Coleoptera, Araneae, and adult Chironomidae. In the studied sites, only a marginal case of piscivory was observed in site D2, where *A. anguilla* preyed on *G. lozanoii* (Table S1.1).

Richness and biomass of detritivore invertebrates decreased drastically by the dam, and recovered downstream (Fig 1.2a). Herbivore invertebrates replaced the detritivore invertebrates at all impact sites (Fig 1.2b). Orthocladinae, the most abundant among the widespread taxa, presented a detritivore diet only at site U1, and shifted to herbivore strategies at the other sites by significantly reducing the ingested detritus fraction (PERANOVA, pseudo- $F_{3,76} = 5.672$, $p = 0.01$; Fig 1.2c). This diet shift was observed for the freshwater limpet *Ancylus fluviatilis* (Planorbidae, Gastropoda) between sites U1 and D3 (t-test, $F_{30} = 13.947$, $p = 0.01$) (see Table S1.1). Chironomidae (Diptera) were

the most abundant and recurrent prey (64.5% in vertebrates, 44% in total invertebrates, 83% in insectivorous invertebrates).

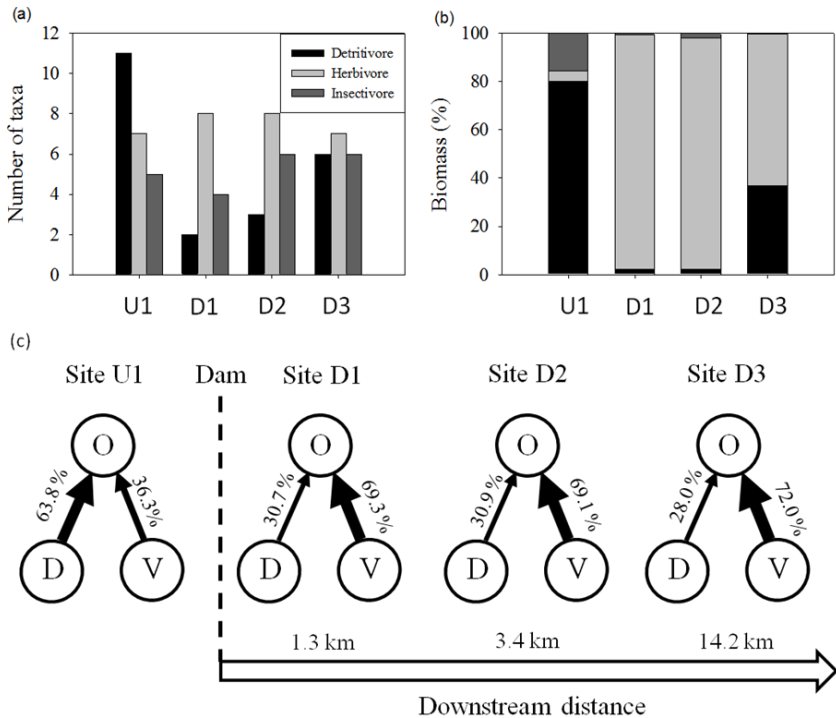


Fig. 1.2 Macroinvertebrate feeding strategies at each study site. (a) richness of each feeding strategy; (b) proportion of biomass of each feeding strategy; (c) Orthocladiinae diet shifts between detritus + dead animal material (“D”) and vegetal material (including diatoms, algae and fungi; “V”), upstream and downstream of the dam. Percentages represent the contribution of each

Invertebrate body size spectra differed among sites (Fig 1.3). Mean invertebrate size was smaller downstream of the dam, in spite of larger individuals occurring in those sites (Table 4). Prey size of invertebrate predators was similar among sites (Table 1.4 and Fig 1.3; PERANOVA, pseudo- $F_{3,182} = 0.386$, $p = 0.77$), but in site U1 the range of their prey size was wider. Invertebrate

predator size was one order of magnitude higher downstream of the dam, and the body mass ratio of invertebrate predator-prey (Table 1.4) was higher at sites D1 and D2 and decreased further downstream (site D3), presenting similar values to those found in the upstream site. The size range of fish prey was highest at site D1, and decreased downstream (Fig 1.4 and Table 1.4).

Network properties

The number of nodes and trophic links (see Table S1.6) in the food webs increased downstream of the dam (Table 1.5). The maximum number of nodes and trophic links was recorded at site D2, where the lowest connectance values and the highest resource:consumer ratios were observed (Table 1.5). The upstream site had a high fraction of top-level species, and of direct trophic interactions between top predators and basal resources occurred (Table 1.5). The sites downstream of the dam presented a large fraction of intermediate species and a wider food web (Fig 1.4, Table 1.5). Mean food chain was longest at site D1, where median and maximum FCL also peaked (Table 1.5). The maximum degree of omnivory was observed at sites D1 and D3, and the number of resources per consumer (generality) increased in all sites downstream of the dam, peaking in D3 (Table 1.5). The standard deviation of the number of consumers per resource (vulnerability) exceeded that of generality in all sites, indicating a greater variability in the number of consumers than of resources for a given species (Table 1.5).

Table 1.4 Invertebrate and vertebrate community structure at each study site. Feeding strategies were assigned after Tachet et al. (2002). Invertebrate sizes are reported in mg of dry weight. “Prey Size of Vertebrate Predators” shows the mean and the range of invertebrate prey consumed by vertebrates.

	Site U1	Site D1	Site D2	Site D3
Invertebrate richness	28	33	28	26
Invertebrate density (ind/m ²)	2140	4864	2034	9332
Non-Orthocladiinae invertebrate abundance (ind/m ²)	936	282	1548	4458
Vertebrate richness	2	6	5	6
Vertebrate density (ind/m ²)	0.03	2.05	2.06	2.24
Community diversity (H')	3.2	2.1	2.7	3
Percentage of Invertebrate Feeding Strategies (%)				
Scraper	42.7	36.4	35.7	38.5
Shredder	25	18.2	10.7	15.4
Predator	7.1	21.2	28.6	15.4
Deposit feeder	10.7	12.1	10.7	15.4
Filter feeder	7.1	6.1	7.1	7.7
Piercer	7.1	6.1	7.1	7.7
Invertebrate Size				
Mean (mg/ind)	1.4 ± 0.1	0.6 ± 0.3	1.0 ± 0.3	0.3 ± 0.02
Range (mg)	0.2·10 ⁻³ – 40.1	7.4·10 ⁻³ – 371.5	7.0·10 ⁻³ – 208.2	1.6·10 ⁻³ – 45.9

Continued on next page

Table 1.4 - Continued from previous page

	Site U1	Site D1	Site D2	Site D3
Size of Invertebrate Predators				
Mean (mg/ind)	3.1 ± 0.7	38.0 ± 28.6	33.5 ± 15.1	15.2 ± 5.6
Range (mg)	0.2 – 12.2	0.7 – 208.2	0.04 – 208.2	0.05 – 45.9
Prey Size of Invertebrate Predators				
Mean (mg/ind)	0.2 ± 0.02	0.2 ± 0.05	0.3 ± 0.06	0.26 ± 0.06
Range (mg)	1.8·10 ⁻³ – 1.2	2.7·10 ⁻³ – 0.8	0.2·10 ⁻³ – 2.8	2.91·10 ⁻³ – 1.3
Invertebrate Predator-Prey mass ratio	41.7 ± 10.3	1541.0 ± 828.4	1206.7 ± 641.3	130.2 ± 22.0
Prey Size of Vertebrate Predators				
Mean (mg/ind)	97.3 ± 158.3	1.94 ± 10.19	0.1 ± 1.9	0.3 ± 2.3
Range (mg)	89.2·10 ⁻⁴ – 341.1	3.2·10 ⁻⁴ – 132.7	0.3·10 ⁻⁴ – 98.4	3.5·10 ⁻⁴ – 42.7

Discussion

The effects of flow regulation by dams on riverine habitat and organisms have long been studied (e.g., Brittain and Saltveit, 1989; Poff and Zimmerman, 2010; Ponsatí et al., 2015), but impacts at the higher levels of biological organization (e.g., food webs) have received relatively less attention (but see Power et al., 1996, and Cross et al., 2013). Here we described longitudinal variation in food-web topology in a dam-regulated intermittent Mediterranean river, and found a positive association between dam-induced flow stability and resource quality, herbivory (over detritivory), and food-chain length and width. The impacts of regulation on food-web structure persisted downstream, despite a partial recovery of the flow regime.

Flow stability and herbivory

Dam-induced hydrological stability promoted the growth of algae over macrophytes, via flood suppression and increased riparian shading (Spink et al., 1993; Janauer and Dokulil, 2006). The higher nutritional quality of algae relative to detritus (Bowen, 1987; Stelzer and Lamberti, 2002), and the associated increase in algal production downstream of dams, can turn detritus-based into algae-based food webs (Power et al., 2013). In our study, the abundant Orthocladiinae and *A. fluviatilis* shifted diets accordingly. Several studies have shown that dams vastly reduce the frequency of high flows, favouring less dynamic hydromorphological conditions downstream (Batalla et al., 2004; Döll et al., 2009). This reduction in the frequency and intensity of floods often results in

the terrestrialization of fluvial systems. This occurred in the Montsant River, where reduction in river width allowed terrestrial vegetation to encroach in part of the streambed. Taken together, these abiotic and biotic changes, ultimately controlled by the flow regime, influenced the type of basal resources in the riverine food web.

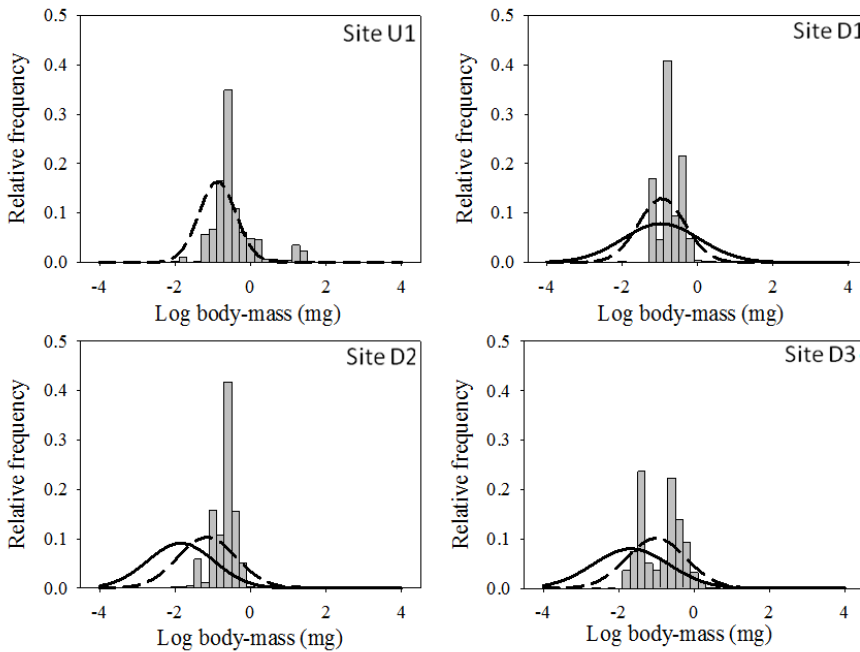


Fig. 1.3 Macroinvertebrate body size distribution at the different study sites. Gray bars represent invertebrate body size availability, discontinuous lines represent invertebrate body size consumed by invertebrates, and continuous lines represent invertebrate body size consumed by fish.

Species properties	Site U1	Site D1	Site D2	Site D3
Number of nodes (S)	41	59	88	59
Number of trophic links (L)	170	283	434	322
Fraction top level	0.4	0.2	0.2	0.2
Fraction intermediate	0.4	0.7	0.8	0.6
Fraction basal	0.2	0.1	0.08	0.1
Ratio resources:consumers	0.7	0.8	0.9	0.9
Link properties (Complexity)				
Link density	4.1	4.8	4.9	5.5
Connectance	0.1	0.08	0.06	0.09
Fraction of links between				
Top and intermediate	0.15	0.13	0.18	0.2
Top and basal	0.26	0.1	0.1	0.11
Intermediate	0.12	0.22	0.2	0.14
Intermediate and basal	0.46	0.55	0.52	0.55
Chain properties				
Mean chain length	2.3	3.9	2.8	2.7
Median chain length	2	3	3	3
SD chain length	0.9	0.9	1	0.9
Maximum chain length	4	5	5	5
Omnivory properties				
Degree of omnivory	0.12	0.17	0.11	0.2
Consumer-prey asymmetries				
Generality	5	5.4	5.4	6.4
Vulnerability	6.8	6.2	5.9	7.4
SD standardised generality	0.7	0.8	1.3	0.9
SD standardised vulnerability	1.7	1.8	2.3	1.8

Table 1.5 Food-web structure metrics at each site. Invertebrate terrestrial prey were excluded from this analysis, but considered in the rest of the study. SD, standard deviation.

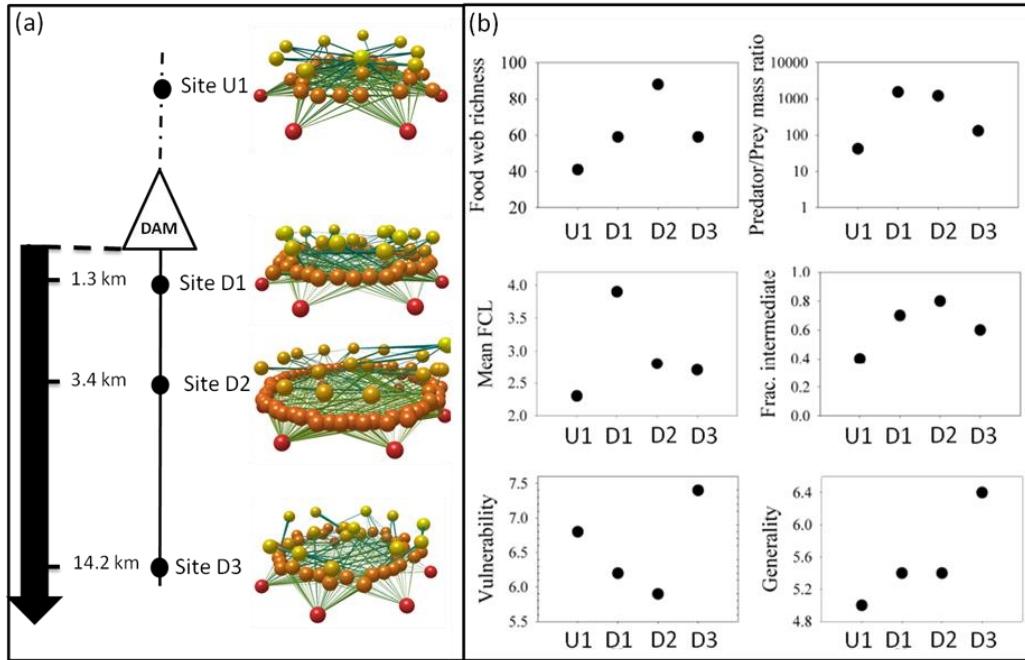


Fig. 1.4 Food-web diagrams and metrics. (a) Food-web diagrams representing basal resources (red), intermediate consumers (orange) and top predators (yellow), and the interactions among them. Diagrams were produced with the Network 3D software (Williams, 2010; Yoon et al., 2004). (b) Food-web structure metrics at each study site: Food-web richness considering all taxa, including those present only in gut contents; mean predator:prey mass ratio (mg/mg); mean food-chain length (an average of the different food chains across all the taxa in each food web); fraction of intermediate taxa (consumer taxa being preyed upon); vulnerability (number of consumers per taxa); and generality (number of resources per taxa).

Terrestrialization and predatory interactions

Terrestrialization occurred in the regulated sites, and was manifested by a higher influence of terrestrial vegetation over the river channel, including the colonization of terrestrial plants on the streambed. This habitat change was associated with stream channel narrowing, and enabled predatory fish to complement their diets with terrestrial invertebrates (particularly in site D2). Terrestrial invertebrates can represent a substantial energy source for stream communities (Nakano et al., 1999a), and this subsidy could have favoured higher fish densities in site D2 (as in Woodward and Hildrew, 2002a). Predation on terrestrial prey could reduce fish pressure on freshwater invertebrates, thus increasing their densities and the fraction of links among intermediate species. These patterns are consistent with the decrease in omnivory and food-web widening observed in these sites. High fish densities supported by terrestrial prey can produce top-down effects if terrestrial intakes are interrupted (as in Nakano et al., 1999b). Thus, dam-induced terrestrial subsidization could decrease long-term stability of the subsidized riverine food web.

Food-web widening and lengthening

Dam-induced hydromorphological stability promoted autochthonous production and decreased allochthonous inputs at the base of the food webs. The availability of higher-quality basal resource downstream of the dam increased the richness and abundance of primary consumers, widening the food web. Several network metrics (species richness, number of links, mean FCL,

and vulnerability) reflected horizontal and vertical changes in food-web structure. FCL results from community membership, available resources, predator-prey interactions, disturbance, and ecosystem size (Post, 2002a). Therefore, although the barrier effect of the dam probably contributed to limiting fish population at the upstream reach (Power and Dietrich, 2002), surface water drying in that site also likely reset the community (Power et al., 2008). Drying limited the presence of viable fish populations upstream of the dam. This likely explains the commonly-observed shorter FCL in intermittent sites (McHugh et al., 2010; John L Sabo et al., 2010). In turn, the change from a detritus-based to an algal-based food web may enhance the abundance and richness of primary consumers. This could increase the abundance of predators and of the interactions among them (i.e., intraguild predation), lengthening FCL downstream of the dam (Ruhí et al., 2016). Of special interest is the decline in connectance in sites D1 and D2, probably related to increases in species richness and generality, in food-chain length (Schmid-Araya et al., 2002; Woodward and Hildrew, 2002b, 2002a), and in body size disparity between invertebrates in the bottom *vs.* top of the food web (Schmid-Araya et al., 2002).

In addition to increased invertebrate richness and density, and decreased individual sizes, invertebrate predators downstream of the dam shifted from being dominated by Plecoptera to being exclusively represented by Odonata. Odonata can be adapted to coexist with fish (Pierce, 1988), and have passive ‘sit-and-wait’

foraging strategies (Tachet et al., 2010). Their elongated masks reduce reactive distances and differences in movement speed between predators and prey, allowing these large-bodied predators to capture smaller prey. This is coherent with the relatively higher predator:prey size ratios observed in the impact sites.

Composition shifts were also observed for vertebrates, with main differences being explained by fish richness and abundance. Unlike in the naturally-intermittent upstream site, downstream of the dam perennial flow sustained fish populations; accordingly, fish predator densities were higher there. Fish predation may have kept at bay amphibian larvae in the impact sites, as described by Hecnar and M'Closkey (1997) from lentic habitats.

Longitudinal patterns in food-web structure

Further downstream of the dam, small intermittent tributaries joined the regulated main stem. This restored the frequency of high and low-flow events observed in D3, but not their magnitude. The partial restoration of the flow regime was associated with an increase in river width, which reversed the terrestrialization observed just below the dam. There was a decrease of local allochthonous inputs at the food-web base, and an increase in light penetration. These changes favoured macrophyte abundance at the most downstream site, although algal-based sources still largely dominated. The reduction in FCL relative to the impacted (hydrologically more stable) upstream reaches indicates that other constraints like extinction-colonization

dynamics could be limiting (Post, 2002a). In this site (D3), macroinvertebrate body size partially recovered and size range was reduced. Schmid-Araya et al. (2002) reported that body-size disparity among organisms at the bottom *vs.* top of the food web could influence connectance. In our case, the reduction of size range allowed an increase in connectance. Additionally, a high number of predators feeding on a given prey is reflected in a higher vulnerability value, a property often associated with keystone species (Calizza et al., 2015). The relatively more unstable conditions in sites U1 and D3 could contribute to the high vulnerability values observed in those sites.

Concluding remarks

Our results illustrate how flow regulation by dams can alter food-web structure in intermittent rivers, not only via changes in community composition but also via changes in the relative importance of autochthonous production *vs.* allochthonous inputs. In the studied Mediterranean river, flow regulation increased basal autochthony and that led to wider and longer food webs. The recovery of network structure downstream of the dam was only partial. Thus, our study advances the notion that serial discontinuity may present cumulative effects on food webs, and impacts of flow regulation by dams may persist even if the physical template is locally restored. Our results emphasize that dam-induced flow alteration can impact the higher levels of biological organization. This is relevant in the light of the steep increase in dam planning and building globally, especially in developing,

highly-biodiverse regions, where water resource and hydropower demand is escalating.

A large concrete pipe is shown discharging water into a brick-lined channel. The pipe is made of several sections and is surrounded by a structure of bricks and concrete. The water is flowing out of the pipe into the channel. The background shows some green vegetation and a clear sky.

CHAPTER 2

Invertebrate community responses to urban wastewater effluent pollution under different hydro-morphological conditions

Mor, J.R., S. Dolédec, V. Acuña, S. Sabater and I. Muñoz.
ENVIRONMENTAL POLLUTION..
2019. **252**, Part A: 483-492.

Background

River systems are increasingly vulnerable to growing water demands and pollution inputs (Vörösmarty et al., 2010). In particular, arid and semiarid rivers can be more sensitive to pollution because urban wastewater effluents cannot be properly diluted (Petrovic et al., 2011; Rice and Westerhoff, 2017). In these arid or semiarid systems and under conditions of low (or absent) basal flow, wastewaters can be the main water source (reaching up to 70 – 100% of the flow; Murdock et al., 2004), therefore strongly affecting the local biological communities (Corcoll et al., 2015; De Castro-Català et al., 2017; Huerta et al., 2015).

Under these circumstances, urban wastewater pollution might adversely affect the most nutrient and pollutant sensitive invertebrate groups, such as Ephemeroptera, Plecoptera and Trichoptera, while favouring pollutant tolerant taxa, such as some Chironomidae and Oligochaeta (Ortiz and Puig, 2007). Urban wastewater pollution may reduce dissolved oxygen in the hyporheic zone, causing immediate changes to the associated meiofauna richness (Sánchez-Morales et al., 2018). This pollution has been also shown to, enhance the abundance of invertebrates larger than 2 cm, as well as the occurrence of plurivoltine taxa (more than one generation per year), and to favour the occurrence of those with tegument respiration and crawlers (Charvet et al., 1998). In addition, urban wastewater pollution enhance the accumulation of fine benthic organic matter, which is currently

associated with an increase in deposit feeders and a decrease in scrapers and shredders (Shieh et al., 1999).

Although the joint occurrence of wastewater effluents and hydrological stress is increasingly common (Sabater et al., 2018), the study of urban wastewater pollution on water stressed regions has received little attention (Arenas-Sánchez et al., 2016). Here, we investigated the effects of these co-occurring stressors by analysing changes on the invertebrate community composition as well as on their functional traits. The analysis was performed on a set of small to medium-sized Mediterranean streams, characterised by a high variability in water flow regimes. We hypothesised that (1) wastewater effluents would be the main factor affecting community composition and functional adaptations of invertebrate assemblages. Given that invertebrate communities are originally adapted to hydrological variability (even intermittency), the hydrological stress on impacted sites should be restricted to a secondary role regarding the increase of the chemical impact during low flow periods. We also expected that (2) the pollutant impact of urban wastewaters would act as an environmental filter, selecting taxa whose functional traits promote greater resistance (i.e., tolerance). Further, (3) whereas macroinvertebrate assemblages are characterised by river intrinsic properties (i.e., discharge, width and substratum), wastewater taxa selection should lead the invertebrate community towards higher taxonomic and functional homogeneity.

Material and Methods

Study location

Three sampling campaigns were performed during summer and autumn 2015 and spring 2016 at twelve sampling locations spread across ten small to medium-sized tributaries of the Ebro River (NE Iberian Peninsula, Fig. 2.1). All the streams studied were situated in low-mountain Mediterranean locations, ranging from 365 to 950 m in altitude, and characterised by dry summer periods with marked flow reductions and rainfall episodes in

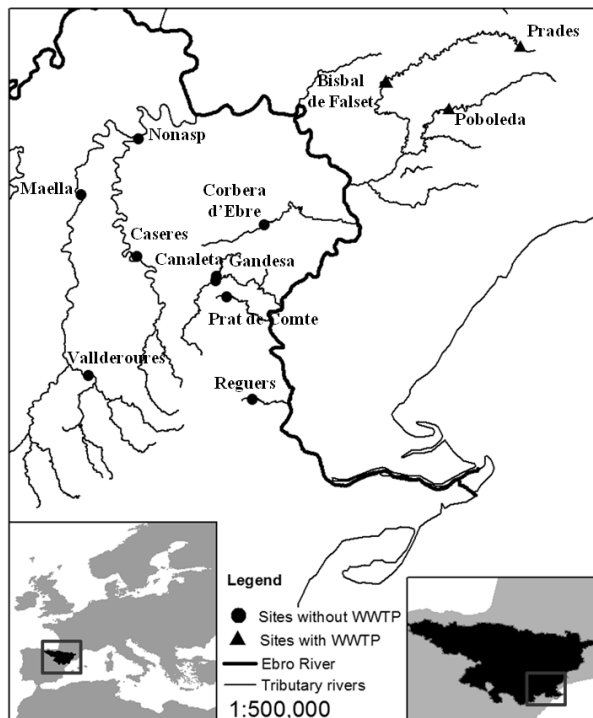


Fig. 2.1 Location of the studied streams in the Ebro catchment. Dots represent untreated wastewater effluents, and triangles wastewater treatment plant (WWTP) effluents.

spring and late autumn. The basins were mainly forested, with low agriculture intensity and pasture activities, and low population density (Table 2.1). In every sampling location, the upstream site was compared to a site placed downstream that received urban wastewater effluents. The downstream sites, fixed over the sampling, had a complete mixture of effluents and stream waters (checked by having a homogenous conductivity across a stream section during the rainy season). The upstream and downstream sites were situated between 200 m and 1300 m from each other, depending on the stream size. Differences in land use were minimal between up and downstream sites and no tributaries or water flow diversions intervened between them. Overall, the only difference between up and downstream sites was the discharge of urban wastewater effluents into the downstream sites. Nine pairs of sites received untreated urban effluents whereas the remaining three received treated effluents from tertiary wastewater treatment plants (Table 2.1; Fig. 2.1). All sites were surveyed to characterise macroinvertebrate assemblages, physical habitat, water chemistry and hydrology.

Stream substrate characterisation

Stream surface sediments in upstream and downstream sites were characterised according to the Wolman pebble count method (Wolman, 1954). We elaborated a grain size curve to determine the D_{50} grain size. Streambed substrates were categorised as sand-dominated in those sampling locations with a $D_{50} \leq 2$ mm and cobble-dominated for those with a $D_{50} \geq 64$ mm.

Stream site	Substrate	Effluent	Resident Population	Upstream basin area (Km ²)	Land use (%)					
					UR	AG	FO	GR	RI	RE
Prades	Sand	WWTP	587	6	4.7	43	48.7	1.3	2.4	0
Bisbal de Falset	Cobble	WWTP	208	132	0.6	22.6	44.8	27.8	4.1	0
Poboleda	Cobble	WWTP	374	123	0.4	23.6	50.3	20.8	4.3	0.6
Nonasp	Cobble	Untreated	992	403	0.1	49.9	33.4	12.3	4.3	0
Caseres	Cobble	Untreated	253	217	<0.1	45.2	39.9	10.7	4.2	0
Maella	Cobble	Untreated	1970	983	0.1	35.8	44.7	15	4.3	0.1
Vallderoures	Cobble	Untreated	2311	205	0.2	12	61.6	21.5	4.3	0.4
Canaletes	Sand	Untreated	615	68	<0.1	28.9	52.9	14	4.2	0
Gandesa	Sand	Untreated	615	29	<0.1	67.6	27.3	1.1	4	0
Prat de Comte	Sand	Untreated	177	9	<0.1	33.7	47.5	14.3	4.5	0
Reguers	Cobble	Untreated	653	27	0.9	24.7	37.2	32.8	4.5	0
Corbera d'Ebre	Sand	Untreated	1080	43	1.5	84.8	9.2	0.4	4.3	0

Table 2.1 Studied sites description. Substrate types (from Table S2.1), effluent types (tertiary wastewater treatment plants (WWTP) and untreated effluents), resident population (2016) and % land use cover upstream at the studied sites (UR = Urbanized, AG = Agricultural, FO = Forested, GR = Grasslands, RI = river, RE = reservoir).

Hydrological characterisation

Water level was measured continuously from April 2015 to May 2016 (at hourly intervals) in the upstream and downstream sites, using level loggers (Solinst Levellogger, Canada). Instantaneous water depth, velocity, and discharge were measured at each sampling campaign by means of a snapshot cross section using an acoustic Doppler velocity meter (ADV; Flow Tracker, SonTek Handheld-AD®, P-4077). Actual discharge data were only used for a characterisation of the sampling date, while water levels of the 60 days prior to each sampling campaign were used to characterise hydrological patterns from the following descriptors:

(1) Tendency to lose or gain water (H_t) during a given period (t , 60 days). The index was calculated as $H_t = ((h_0 - h_t) / h_t) / C_v$, which uses the slope between the mean water level on the sampling day (h_0) and the mean water level of t days before the sampling day (h_t), divided by the coefficient of variation of the water level data (C_v). $H_t > 0$ values indicated that the sampling location was gaining water during the period t , while $H_t < 0$ values indicated that it was losing water during the period t .

(2) Long-term water level differences (LTWD) were estimated as the difference between the mean water level for the selected period (h_{pt} , 60 days) and the mean annual water level, and estimated as $LTWD_{pt} = \overline{h_{pt}} - \overline{h_L}$. $LTWD_{pt} > 0$ values indicated that the location had a higher water level during the considered period (pt) than during the extended period (L). Values of $LTWD_{pt} < 0$

indicated that the location had a lower water level during *pt* than during *L*.

(3) Duration of a low water level was calculated as the sum of hours (expressed in days) having a water level below 0 cm (dry riverbed), 3 cm and 5 cm for the 60 days before the sampling.

Physical-chemical characterisation

Water temperature ($^{\circ}\text{C}$) was measured hourly, from April 2015 to May 2016, with data-logger (Solinst Levelogger, Canada). Water pH, dissolved oxygen, and electrical conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$) were measured *in situ* using hand-held probes (WTW, Weilheim, Germany). Water samples for phosphate ($\mu\text{g P}\cdot\text{PO}_4^{3-}\cdot\text{L}^{-1}$), total phosphorous ($\mu\text{g P}\cdot\text{L}^{-1}$), nitrite ($\mu\text{g N}\cdot\text{NO}_2^{-}\cdot\text{L}^{-1}$), nitrate ($\mu\text{g N}\cdot\text{NO}_3^{-}\cdot\text{L}^{-1}$), ammonium ($\mu\text{g N}\cdot\text{NH}_4^{+}\cdot\text{L}^{-1}$), total nitrogen (TN, $\mu\text{g N}\cdot\text{L}^{-1}$) and DOC concentrations (DOC, $\mu\text{g C}\cdot\text{L}^{-1}$) were collected at each site (upstream and downstream) and analysed. Water temperature ($^{\circ}\text{C}$) was measured hourly from April 2015 to May 2016 with data-logger (Solinst Levelogger, Canada). Water pH, dissolved oxygen, and electrical conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$) were measured *in situ* using hand-held probes (WTW, Weilheim, Germany). Water samples for chemical analyses were collected at each site (upstream, downstream) and sampling campaign, filtered on $0.7\ \mu\text{m}$ GF/F filters (Whatman Int. Ltd., Maidstone, UK) and kept at $-20\ ^{\circ}\text{C}$ until analysis. Phosphate ($\mu\text{g P}\cdot\text{PO}_4^{3-}\cdot\text{L}^{-1}$) and total phosphorous ($\mu\text{g P}\cdot\text{L}^{-1}$) concentrations were determined colorimetrically after Murphy and Riley (1962) using a spectrophotometer (Alliance-AMS Smartchem 140, AMS,

Frepillon, France). Nitrite, nitrate and ammonium nitrogen concentrations ($\mu\text{g N-NO}_3^- \cdot \text{L}^{-1}$; $\mu\text{g N-NO}_2^- \cdot \text{L}^{-1}$; $\mu\text{g N-NH}_4^+ \cdot \text{L}^{-1}$) were determined using a Dionex ICS-5000 ion chromatograph (Dionex Co., Sunnyvale, USA; Hach, 2002). DOC concentration ($\text{DOC}, \mu\text{g C} \cdot \text{L}^{-1}$) and total nitrogen (TN, $\mu\text{g N} \cdot \text{L}^{-1}$) were determined with a Shimadzu TOC-V CSH coupled to a TNM-1 module (Shimadzu Co., Kyoto, Japan). The DOC samples in the first sampling campaign are not available.

Pharmaceutical products (Phacs) were assumed to be the most relevant discharge, given the urban origin of the effluents. Phacs concentrations were determined from simultaneous sampling (Mandarić et al. 2018). By using the EC_{50} toxicity values for aquatic invertebrates (Kuzmanovic et al., 2015), pharmaceutical compounds were classified as suggested by Hernando et al., 2006, as: “very toxic” ($\text{EC}_{50} < 1 \text{ ng} \cdot \text{L}^{-1}$); “toxic” (EC_{50} from $1 \text{ ng} \cdot \text{L}^{-1}$ to $10 \text{ ng} \cdot \text{L}^{-1}$); “harmful toxic” (EC_{50} from $10 \text{ ng} \cdot \text{L}^{-1}$ to $100 \text{ ng} \cdot \text{L}^{-1}$) and “slightly toxic” ($\text{EC}_{50} > 100 \text{ ng} \cdot \text{L}^{-1}$). Total concentration for each category was calculated by the sum of the compound concentrations in each group.

Macroinvertebrate sampling

Five sample units from each site were collected randomly using a Surber net (30 x 30 cm, mesh size 250 μm) in cobble-dominated streams or using a core sampler ($\text{Ø} = 25 \text{ cm}$) in sand-dominated stream sites (see below for their definition; Rodrigues-Capítulo et al., 2009). The samples were preserved in 4% formalin, and invertebrates were sorted, counted and identified at the lowest

possible taxonomic level, mostly genera. The first twenty-five randomly collected individuals of each taxon were measured using an ocular reticule (± 0.1 mm), from which biomass (mg dry weight) was derived using published length-mass relationships (Benke et al., 1999; Burgherr and Meyer, 1997; Meyer, 1989; Ramsay et al., 1997; Sabo et al., 2002).

Data analysis

Environmental data (nutrients, pharmaceuticals and hydrological variables) were checked for normality and homoscedasticity, and log-transformed where necessary. Variables showing Spearman correlation values >0.6 were removed to avoid multi-collinearity problems. As a result, NH_4^+ , “very toxic”, “toxic” and “harmful toxic” pharmaceuticals were retained, together with DLMH_{60} and H_{60} , to be used in subsequent analyses.

Community abundances were log-transformed to reduce numerical disparities. Abundance, taxonomic and EPT (Ephemeroptera, Plecoptera and Trichoptera) richness, and taxonomic diversity (alpha-diversity, Shannon, 1949) were calculated for the invertebrate assemblages of each reach. Sixty trait categories (Table S2.2) were obtained from public databases for European macroinvertebrate taxa (mainly at the genus level; Tachet et al., 2010), with some adaptations for the Mediterranean region (Bonada et al., 2007; Bonada and Dolédec, 2011). Taxon affinity for each trait category was quantified using fuzzy coding and further used as a profile (see Chevenet et al., 1994). We then multiplied the frequency of each trait category by the relative log-

transformed abundances of taxa in the sites. The resulting traits-by-site array comprised the relative utilisation of each trait category in each site. Data in the array were arcsine-square root transformed to improve normality distributions (Ahrens et al., 1990).

The environmental differences between substrata types were analysed by each variable and period in the upstream sites using one-way ANOVA (Miller, 1966; Yandell, 1997). Environmental and community differences between up- and downstream sites and between sampling periods were examined using linear mixed models (LMM; Laird and Ware, 1982), where “*sampling location*” was used as a random variable. Statistical significances were tested with Tukey’s HSD test, which compares the obtained model with the null hypothesis of no difference (Bretz et al., 2010; Hothorn et al., 2008). Note that sampling locations at upstream sites that were dry during a given sampling period were not considered in the statistical analysis; this was the case with the Reguers for the three sampling campaigns, and Poboleda during summer 2015. The dissimilarities in community compositions within up and within downstream sites were tested using Bray-Curtis, abundance data, and Sorensen, presence-absence data, beta diversities (Baselga, 2017, 2010). A similarity percentage test (SIMPER) was performed with a cut-off for low contribution at 90% (Clarke, 1993), in order to account for species with higher dissimilarity between up and downstream sites and periods. Standardised major axis (SMA) slopes (Warton et al. 2006) were used to compare the relationship between trait frequency

utilisation in wet and dry periods, at both u- and downstream sites. Dissimilarities in the communities' functional traits were tested using the Edwards distance measure (Edwards, 1971). Environmental relationships with invertebrate assemblage descriptors (compositional and functional) were finally tested using co-inertia analysis. This multivariate analysis allows studying the co-structure between environmental and community data by deriving axes in each dataset that have the maximal covariance (Dolédec and Chessel, 1994).

The SIMPER analysis was performed using PRIMER v.6.0 for Windows whereas all other analyses were performed using the R software (R Core Team, 2014). Co-inertia analyses were computed with the *ade4* (Chessel et al., 2004; Dray et al., 2007; Dray and Dufour, 2007). LMMs were conducted using the *lme4* package (Bates et al., 2015). Marginal and conditional R-squared values were computed using the *MuMIn* package (Barton, 2018) and their significance analysed with *multcomp* package (Hothorn et al., 2008). SMA slope differences were tested with *smart* package (Warton et al. 2012). Beta diversity was calculated using *betapart* package (Baselga et al., 2017). Functional diversity used the *FD* package (Laliberté et al., 2014; Laliberté and Legendre, 2010).

Results

The locations studied encompassed a variety of hydrological characteristics and stream substrata types. One of the sites was ephemeral, three were intermittent (flow stopping in summer) and eight were permanent (Tables 2.2 and S4). The streambed substrata of seven locations were mostly cobbles ($D_{50} > 64$ mm; hereafter named cobble-dominated streams, CdS), with fine sediment (< 2 mm) accounting for $< 10\%$ of surface coverage. Mean width was equal to 7.3 ± 4.5 m, and mean flow discharge equalled 0.28 ± 0.38 m³·s⁻¹ (Tables 2.2 and S5). The remaining five locations (hereafter named sand-dominated streams, SdS) had streambed substrata composed mainly of sand ($D_{50} < 2$ mm). They were generally smaller (width 2.4 ± 1.4 m) and had a lower discharge (0.03 ± 0.04 m³·s⁻¹; Tables 2.2 and S5) than CdS. These variables were used to categorise the two stream types.

Hydrological characterisation

All the locations studied presented a flow contraction (H_{60}) in summer 2015 and a flow expansion in autumn 2015 and spring 2016. Autumn showed a lower water level than the annual mean ($LTWD_{60}$); this period was the driest due to the late arrival of autumnal rains. CdS presented higher discharges than SdS (ANOVA, $F_{1,30} = 10.470$, $p < 0.01$) but the estimated hydrological variables were not significantly different between the two stream types (H_{60} : $F_{1,30} = 0.289$, $p = 0.595$; $LTWD_{60}$: $F_{1,30} = 0.084$, $p = 0.773$).

Table 2.2 Studied sites environmental conditions. Substrate types (from Table S2.1), Stream hydrology and % of dry days (from table S2.3) and mean nutrient ($\text{mg}\cdot\text{L}^{-1}$) and pharmaceutical ($\text{ng}\cdot\text{L}^{-1}$) concentrations (from Table S2.5).

Sampling location	Site	Subs	Stream hydrol.	% of Dry days	N-NH ₄ ⁺	N-NO ₃ ⁻	PT	Very toxic	Toxic	Harmful Toxic
Bisbal de Falset	Up	C	Perm	0	< 0.01	0.22 ± 0.11	0.01 ± 0.00	0.08 ± 0.14	10.41 ± 6.19	12.90 ± 7.49
	Down	C	Perm	0	0.02 ± 0.01	0.22 ± 0.06	0.04 ± 0.02	0.61 ± 0.63	23.65 ± 14.67	40.17 ± 8.60
Canaleta	Up	S	Perm	0	< 0.01	0.86 ± 0.56	< 0.01	0.08 ± 0.14	12.23 ± 12.03	9.84 ± 10.03
	Down	S	Perm	0.02	0.92 ± 1.52	1.03 ± 0.19	0.07 ± 0.06	0.95 ± 1.23	684.83 ± 320.72	43.84 ± 30.72
Caseres	Up	C	Perm	0	< 0.01	5.32 ± 3.18	< 0.01	0.18 ± 0.16	7.02 ± 4.91	9.47 ± 2.73
	Down	C	Perm	0	0.01 ± 0.01	5.21 ± 3.00	0.01 ± 0.00	0.45 ± 0.29	213.24 ± 66.26	44.14 ± 29.20
Corbera d'Ebre	Up	S	Perm	0	< 0.01	8.00 ± 2.72	0.70 ± 0.24	6.37 ± 2.64	121.83 ± 37.13	281.88 ± 58.80
	Down	S	Perm	0	2.49 ± 1.18	7.86 ± 0.86	1.01 ± 0.40	33.3 ± 14.8	3465.03 ± 562.08	2462.75 ± 1501.14
Gandesa	Up	S	Perm	0	< 0.01	0.64 ± 0.33	< 0.01	0.18 ± 0.31	4.02 ± 1.97	7.30 ± 1.24
	Down	S	Perm	0	1.49 ± 1.70	0.74 ± 0.20	0.13 ± 0.12	10.1 ± 8.44	1081.04 ± 698.47	1364.88 ± 1262.97
Maella	Up	C	Perm	0	< 0.01	4.65 ± 2.42	< 0.01	< 0.01	5.80 ± 2.34	10.10 ± 3.18
	Down	C	Perm	0	2.64 ± 2.54	1.22 ± 0.24	0.34 ± 0.27	9.49 ± 14.5	146.99 ± 70.21	237.67 ± 212.55
Nonasp	Up	C	Perm	0	< 0.01	1.81 ± 1.46	< 0.01	0.02 ± 0.03	14.82 ± 11.79	10.33 ± 2.54
	Down	C	Perm	0	0.58 ± 0.96	1.45 ± 1.64	0.08 ± 0.09	0.50 ± 0.39	205.96 ± 115.57	1290.94 ± 724.11

Continued on next page

Table 2.2 - Continued from previous page

Sampling location	Site	Subs	Stream hydrol.	% of Dry days	N-NH ₄ ⁺	N-NO ₃ ⁻	PT	Very toxic	Toxic	Harmful Toxic
Poboleda	Up	C	Inter	2.57	< 0.01	0.01 ± 0.00	0.01 ± 0.00	< 0.01	4.25 ± 2.53	5.43 ± 5.38
	Down	C	Perm	0.09	0.11 ± 0.16	0.11 ± 0.09	0.04 ± 0.02	2.30 ± 3.26	91.28 ± 48.37	135.91 ± 67.01
Prades	Up	S	Perm	0	0.01 ± 0.00	2.30 ± 0.60	0.04 ± 0.00	< 0.01	7.16 ± 8.34	11.97 ± 4.31
	Down	S	Perm	0	4.20 ± 2.12	1.81 ± 1.20	0.61 ± 0.44	11.7 ± 10.9	137.69 ± 57.55	244.30 ± 59.26
Prat de Comte	Up	S	Inter	7.08	< 0.01	3.24 ± 0.60	0.01 ± 0.00	0.41 ± 0.37	16.28 ± 8.37	11.56 ± 8.64
	Down	S	Perm	0	3.36 ± 2.13	1.53 ± 0.15	0.35 ± 0.24	64.5 ± 66.5	7315.66 ± 511.43	556.12 ± 380.16
Reguers	Up	C	Ephe	99.49	-	-	-	-	-	-
	Down	C	Inter	2.57	-	-	-	-	-	-
Vallderoures	Up	C	Perm	0	< 0.01	1.37 ± 1.10	< 0.01	0.16 ± 0.14	13.65 ± 5.83	17.63 ± 13.80
	Down	C	Perm	0	0.41 ± 0.14	1.64 ± 1.16	0.10 ± 0.03	2.25 ± 2.64	394.23 ± 200.53	394.51 ± 243.21

The up and downstream sites did not show significant temporal differences in water levels or instantaneous water flow. Thus, the hydrological variables H_{60} or $LTWD_{60}$ were not significantly different (LMM, Tukey's HSD test: $p = 0.855$ and $p = 0.897$ respectively; Table S2.4 and S2.5). The discharges associated with wastewater effluents did not produce a measurable hydrological alteration in the downstream sites. An exception concerned sampling locations on smaller streams, at which wastewater inputs contributed to basal flow when the water level was < 3 cm (dry periods), thus reducing the number of days with a dry streambed (Table S2.3).

Water chemical characteristics and wastewater effects across sites

Nutrient concentrations in the upstream sites ranged from 0.001-0.022 $\text{mg}\cdot\text{L}^{-1}$ N-NH_4^+ , 0.002-0.029 $\text{mg}\cdot\text{L}^{-1}$ N-NO_3^- , 0.002-0.751 $\text{mg}\cdot\text{L}^{-1}$ phosphate P-PO_4^{3-} and 0.62-8.78 $\text{mg}\cdot\text{L}^{-1}$ dissolved organic carbon. The mean total pharmaceutical concentration in the upstream sites was of 6.53 ± 7.92 $\text{ng}\cdot\text{L}^{-1}$ (Table 2.3), with the exception of the site at Corbera d'Ebre (435 to 1079 $\text{ng}\cdot\text{L}^{-1}$ pharmaceutical products), which received an upstream wastewater discharge (Table 2.2). Harmful toxic pharmaceutical were the most abundant at the upstream sites. Higher upstream values occurred at the SdS than CdS sites (Table 2.3) for: very toxic pharmaceutical concentrations (ANOVA, $F_{1,30} = 3.94$, $p < 0.1$), N-NH_4^+ (ANOVA, $F_{1,30} = 11.84$, $p < 0.001$) and P-PO_4^{3-} ($F_{1,30} = 10.75$, $p < 0.001$). These higher values were mainly due to the polluted

upstream site at Corbera d'Ebre (Tables 2.2 and S7). All other parameters showed no differences between upstream sites.

The downstream CdS sites had N-NH_4^+ and P-PO_4^{3-} concentrations fourfold higher than the upstream sites (LMM, Tukey's HSD test, $p < 0.001$; Table S2.4), but N-NO_3^- concentrations remained similar ($p = 0.52$; Table 2.3 and Table S2.4). Total pharmaceutical product concentrations reached up to $2502.95 \pm 2891.11 \text{ ng}\cdot\text{L}^{-1}$ (Tables 2.2 and S7). The very toxic pharmaceutical products in the downstream CdS had higher concentrations during autumn ($p < 0.001$; Table S2.4), while higher values occurred in autumn and spring in the downstream

	Variable	All Up	All Do	CdS Up	CdS Do	SdS Up	SdS Do
Hydrolog	LTWD ₆₀	0.01	0.01	0.01	0.01	0.005	0.003
	H ₆₀	-2.16	-1.50	-0.22	-0.43	-4.16	-2.56
	Discharge	0.06	0.06	0.11	0.104	0.011	0.021
Nutrients	NH_4^+	0.005	1.52	0.004	0.791	0.009	2.496
	NO_3^-	2.67	2.14	2.235	1.661	3.013	2.597
	NO_2	0.006	0.076	0.005	0.067	0.007	0.084
	PO_4	0.045	0.11	0.004	0.097	0.132	0.345
	PT	0.08	0.27	0.011	0.152	0.156	0.44
	TN	2.91	4.52	2.517	2.985	3.39	6.152
	DOC	5.04	5.67	1.865	2.378	8.21	9.055
Pharmaceuticals	Very Toxic	0.70	12.72	0.08	2.62	1.41	24.16
	Toxic	20.26	1287.12	9.63	184.4	32.31	2536.56
	Harmful	36.24	634.69	11.31	370.24	64.51	938.38

Table 2.3 Hydrological, nutrients and pharmaceutical mean values for cobble-dominated streams (CdS, N=18) and sand-dominated streams (SdS, N=15). Up = upstream, Do = Downstream. Units: discharge ($\text{m}^3\cdot\text{s}^{-1}$); Nutrients ($\text{mg}\cdot\text{L}^{-1}$); Pharmaceuticals ($\text{ng}\cdot\text{L}^{-1}$).

SdS ($p < 0.001$; Tables S4 and S5). Downstream SdS had the highest nutrient concentrations as well as the highest mean concentrations of pharmaceutical products (Table 2.3). Higher N-NH₄⁺ concentrations (Tukey's HSD test, $p < 0.05$; Table S2.4) as well as toxic and harmful toxic ($p < 0.001$ and $p < 0.05$, respectively; Table S2.4) pharmaceutical products were consistent across the three sampling periods.

Macroinvertebrate assemblages in the upstream sites

The taxonomic richness of upstream macroinvertebrate communities ranged from 14 to 31 taxa (mean: 21.2 ± 4.8) in CdS. The SdS had a lower taxonomic richness (ANOVA, $F_{1,30} = 32.712$, $p < 0.001$) that ranged from 1 to 19 taxa (mean: 8.5 ± 6.7). SdS also had lower values of EPT richness than CdS ($F_{1,30} = 31.73$, $p < 0.001$; mean 2.6 ± 3.1 and 8.9 ± 3.2 respectively) and lower taxonomic diversity ($F_{1,30} = 16.65$, $p < 0.001$; Fig. 2.2a; mean 3.1 ± 0.6 and 2.1 ± 0.8 , respectively; Table 2.4). The CdS presented 50.2% of taxonomic similarity, being Chironomidae, Caenidae, Elmidae, Baetidae and Corixidae prominent in these sites. The upstream SdS (Table 2.5) had a 33.5% average similarity between the sites, these being dominated by Chironomidae.

Stream type	Site	Richness	EPT Richness	Diversity (H')
CdS	Up	25.7 ± 5.7	8.9 ± 3.2	3.1 ± 0.6
	Down	18.9 ± 6.4	0.7 ± 4.0	2.2 ± 0.6
SdS	Up	12.3 ± 7.5	2.6 ± 3.1	2.1 ± 0.8
	Down	8.1 ± 4.9	0.6 ± 1.4	1.5 ± 0.8

Table 2.4 Mean macroinvertebrate richness, Ephemeroptera, Plecoptera and Trichoptera (EPT) richness, and diversity.

CdS upstream sites had a higher proportion of individuals smaller than 5 mm than those in SdS. The range expanded to 40 mm in SdS, given the higher presence of Glossiphoniidae and Lumbriculidae. Overall, the invertebrates inhabiting CdS presented higher diversity in twelve functional trait categories than those living in SdS ($F_{1,62} = 6.713$, $p < 0.05$). Invertebrates in CdS were characterized by shorter life durations ($F_{1,30} = 7.94$, $p < 0.01$) and aerial active dispersal ($F_{1,30} = 11.09$, $p < 0.01$) whereas aquatic passive dispersal was more frequent in the SdS ($F_{1,30} = 6.62$, $p < 0.05$). The occurrence of diapause was the most common resistance strategy in the invertebrates inhabiting the CdS (e.g., Elmidae, Coleoptera) whereas those producing cocoons (e.g., Oligochaeta) were more common in SdS ($F_{1,30} = 13.84$, $p < 0.001$). Gills was the common respiration type in CdS ($F_{1,30} = 24.79$, $p < 0.001$) whereas tegument respiration was more frequent in SdS ($F_{1,30} = 13.39$, $p < 0.001$). Surface swimmers (e.g., Gerridae, Heteroptera; $F_{1,30} = 9.74$, $p < 0.01$), burrowers (e.g., Tubificidae; $F_{1,30} = 11.95$, $p < 0.01$) and taxa with interstitial instars (e.g., Haplotaxidae; $F_{1,30} = 10.07$, $p < 0.01$) were more frequent in SdS whereas organisms crawling on the substrate were more common in CdS ($F_{1,30} = 11.18$, $p < 0.01$). CdS mainly hosted shredders and scrapers whereas deposit-feeders prevailed in SdS ($F_{1,30} = 9.71$, $p < 0.01$). Finally, predators were more frequent in CdS than in SdS ($F_{1,30} = 10.22$, $p < 0.001$).

Wastewater effects on macroinvertebrate community structure and functioning

Taxonomic and EPT richness, and taxonomic diversity decreased in the downstream sites, irrespective of the stream type (LMM, Tukey's HSD test, $p < 0.05$; Table S2.4). However, the abundance of individuals increased in the downstream sites (CdS, $p < 0.01$, SdS, $p = 0.069$; Tables S6 and S8). Wastewater pollution effects on invertebrate community composition were mainly related to the species turnover ($p < 0.001$ in both stream types; Table S2.4). Ephemeroptera, Trichoptera and Coleoptera taxa decreased whereas Diptera and Gasteropoda increased, in the downstream sites (Table 2.5). Chironomidae, Caenidae, Baetidae and Physidae were dominant in CdS and provided 49.2% of average similarity among downstream reaches. Chironomidae, Simuliidae and Psychodidae prevailed in SdS (41.3% of average similarity). Changes in the communities' structures were significantly related to the high values of pharmaceuticals and nutrients in the downstream sites (co-inertia analyses: CdS, $R^2 = 0.429$, $p = 0.001$; SdS, $R^2 = 0.407$, $p = 0.003$). In addition, in CdS, the above-observed changes in community structure were also significantly related to $LTWD_{60}$. This was mainly explained by the higher abundance of Caenidae and Baetidae taxa in CdS when these streams have lower discharge values than the annual mean ($LTWD_{60}$).

Characteristic families	Cobble-dominated Streams	Sand-dominated Streams
Upstream	Chironomidae, Caenidae, Elmidae, Baetidae, Corixidae, Hydropsychidae, Hydroptilidae, Simuliidae, Leptophlebiidae, Dugesiiidae, Philopotamidae and Ancylidae	Chironomidae, Baetidae, Leptophlebiidae, Glossiphoniidae and Caenidae
Downstream	Chironomidae, Caenidae, Baetidae, Physidae, Elmidae, Simuliidae, Glossiphoniidae, Dugesiiidae and Hydroptilidae	Chironomidae, Simuliidae, Psychodidae
Changes from upstream to downstream	Decrease of: Elmidae, Caenidae, Baetidae, Corixidae, Hydroptilidae, Hydropsychidae, Philopotamidae, Leptophlebiidae, Ceratopogonidae and Gomphidae Increase of: Physidae, Simuliidae, Chironomidae, Dugesiiidae, Glossiphoniidae, Ancylidae and Lymnaeidae	Decrease of: Baetidae, Caenidae, Leptophlebiidae, Glossiphoniidae, Hydrobiidae, Lymnaeidae, Haliplidae Increase of: Chironomidae, Simuliidae, Psychodidae, Physidae, Culicidae, Stratiomyidae, Empididae, Syrphidae and Dytiscidae

Table 2.5 Macroinvertebrate SIMPER analysis results. Macroinvertebrates families accounting for 90% of similarity and dissimilarity (SIMPER analysis) in the studied sites. Families in the upstream CdS represented 50.2% of the average similarity whereas in the upstream SdS they represented 33.5% of average similarity. Families of the downstream CdR represented 41.19% of the average similarity and a 41.28% in the downstream SdS. Differences between upstream and downstream sites accounted a 59.23% of dissimilarity in CdS and 67.24% in SdS.

The invertebrate community structures in CdS were less similar in the downstream than in upstream sites (Bray-Curtis β -diversity: ANOVA, $F_{1,32} = 14.674$, $p < 0.001$; Fig. 2.2b). This pattern did not occur in the SdS (Bray-Curtis β -diversity: $F_{1,28} = 0.257$, $p = 0.616$; Fig. 2.2b). The dissimilarities between the downstream sites were more evident when taxa presence was considered instead of abundance values (Sorensen β -diversity: CdS, $F_{1,32} = 16.870$, $p < 0.001$; SdS, $F_{1,28} = 3.431$, $p = 0.074$). However, this β -diversity change was not related to a significant change in the turnover or nestedness components (turnover: CdS, $F_{1,32} = 0.217$, $p = 0.644$; SdS, $F_{1,28} = 0.520$, $p = 0.477$; nestedness: CdS, $F_{1,32} = 1.723$, $p = 0.199$; SdS, $F_{1,28} = 0.747$, $p = 0.395$).

The relative utilisation of six trait categories changed significantly more in the downstream than upstream sites in all wastewater pollution impacted sites (Table 2.6). Small-sized organisms (body size < 2.5 mm) and those having semivoltine cycles, eggs at aquatic stage, resistance forms, or gill respiration were less frequent, whereas those having free clutches were more common. Additionally to these common changes, the relative utilisation of up to 35 trait categories significantly changed in the CdS. For instance, all categories related to reproduction significantly decreased or increased in CdS, while in SdS only free clutches significantly increased (Table 2.6). These functional trait changes in CdS were related to the co-occurring pharmaceutical and nutrient concentrations (co-inertia: $R^2 = 0.464$; $p = 0.001$). In contrast, we found no such relationships in the SdS (co-inertia: R^2

= 0.166; $p = 0.262$). Functional diversity was also lower in the downstream sites (LMM, Tukey's HSD test, CdS, $p < 0.001$; SdS, $p = 0.050$, Table S2.4; Fig. 2.2c). The differences in functional trait categories' use among downstream sites were higher than the upstream sites, but were only significant in the CdS (Edwards dissimilarity, ANOVA, $F_{1,32} = 11.468$, $p < 0.01$; Fig. 2.2d).

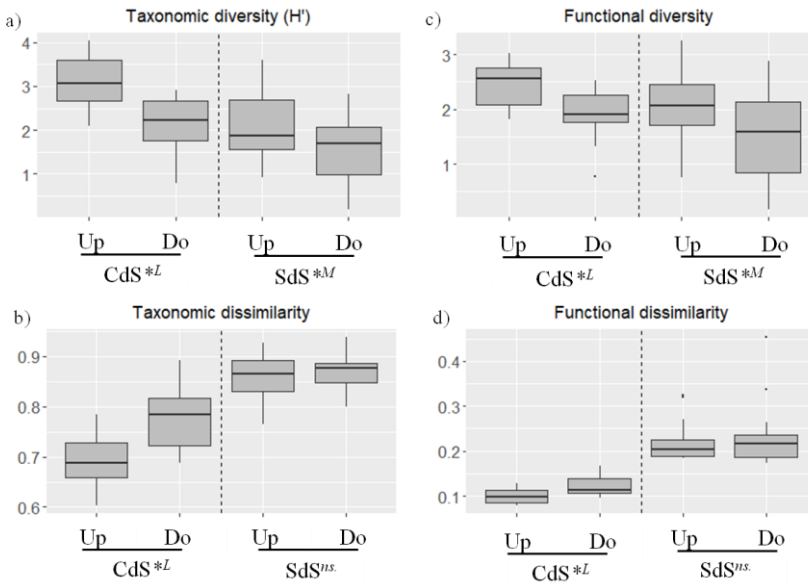


Fig. 2.2 Shannon taxonomic alpha diversity (a), Bray-Curtis taxonomic beta diversity (b), Rao functional diversity (c) and Edwards-distance functional beta diversity (d) in cobble-dominated streams (CdS) and sand-dominated streams (SdS) in upstream (Up) and downstream (Do) sites. Significant differences ($p < 0.05$) are represented by (*), no-significant differences (**). (L) Large Cohen D index effect-size (> 0.8); (M) medium Cohen D index effect-size (0.5-0.8), as defined by Cohen (1992).

Wastewater effect variations between hydrological periods

Dry periods (summer and autumn 2015) had lower dilution capacity than spring 2016 (Table S2.5). As an extreme hydrological situation, the Reguers upstream site regularly dried out and was fed downstream by water coming from the wastewater (untreated) effluent. In that reach, the macroinvertebrate assemblage comprised Diptera (Culicidae, Psychodidae, Syrphidae and Anthomyiidae) and Acheta (Glossiphoniidae; Table 2.5). Another upstream site (Poboleda) dried out during summer and this caused a lower richness (11 taxa) in the downstream site in summer than in autumn or spring (28 and 22 taxa), when treated effluent discharges were diluted.

The situation was less extreme in the remaining locations where water flow was maintained, at least during the sampling periods. In these cases downstream taxonomic diversity decreased during the wet period in comparison to the dry periods (CdS, decreased from 3.28 ± 0.68 to 2.78 ± 0.28 ; SdS, decreased from 2.11 ± 0.79 to 2.07 ± 0.86). The proportion of species common to both up and downstream sites remained higher during the wet period (wet, 53.2 %; dry, 47.5 %) in CdS, but this was reversed in SdS (dry periods, 31.7 %; wet period, 26.7 %). CdS upstream sites showed no difference in functional responses between dry and wet periods (Fig. 2.3a; SMA slope, $p = 0.450$). In contrast, functional trait utilisation in CdS downstream sites differed between dry and wet periods (Fig 2.3a; SMA slope, $p < 0.001$). However, this change

Table 2.6 Wastewater effects on traits categories. Cobble-dominated streams (CdS) and sand-dominated streams (SdS). Arrows indicate the increase or decrease in the relative utilization of each trait category between upstream and downstream sites. Marginal differences ($p < 0.06$) are shown with 0.05 threshold value. Trait-categories with non-significant differences in both river types are not included in this table but could be found at Table S2.6 and S7. Analyses were done with LMM Tukey's HSD tests; estimate, Standard error, Z-value and p value are shown in Table S2.6 and S2.7. Observed responses in previous works were added for comparison Charvet et al. (1998) (a), Shieh et al. (1999) (b).

Trait	Category	CdS	SdS	Previous works
Maximum potential size (mm)	≤2.5	<0.001 (↓)	<0.05 (↓)	(↑) a
	5-10	<0.001 (↑)	ns.	
	10-40	ns.	ns.	
Life duration (y.)	≤1	<0.01 (↓)	ns.	
	>1	<0.01 (↓)	ns.	
Maximum number of reproductive cycles per year	<1	<0.001 (↓)	<0.05 (↓)	(↑) a
	1	<0.01 (↓)	ns.	
	>1	<0.001 (↑)	ns.	
Aquatic stages	Egg	<0.001 (↓)	<0.05 (↓)	
	Larva	<0.001 (↑)	ns.	
	Nymph	<0.01 (↑)	ns.	
	Imago	<0.01 (↓)	ns.	
Reproductive technique	Ovoviviparity	<0.01 (↑)	ns.	
	Isolated eggs, free	<0.01 (↓)	ns.	
	Isolated eggs, cemented	<0.01 (↓)	ns.	
	Clutches, fixed	<0.01 (↓)	ns.	
	Clutches, free	<0.001 (↑)	<0.05 (↑)	
	Clutches in vegetation	<0.05 (↓)	ns.	
Dispersal	Asexual	<0.001 (↑)	ns.	
	Aquatic active	0.05 (↑)	0.05 (↓)	
	Aerial passive	<0.05 (↓)	ns.	
Resistance forms	Aerial active	0.05 (↓)	ns.	
	Eggs, statoblasts and gemmules	<0.01 (↓)	<0.05 (↓)	
	Cocoons	<0.001 (↑)	ns.	
	Cells against desiccation	<0.01 (↓)	ns.	
	None	<0.001 (↓)	ns.	

Continued on next page

Table 2.6 - Continued from previous page

Trait	Category	CdS	SdS	Previous works
Respiration of aquatic stages	Tegument	<0.001	(↑) ns.	(↑) a
	Gill	<0.001	(↓) <0.05	
Locomotion and attachment to substrate	Flier	<0.001	(↓) ns.	(↑) a
	Surface swimmer	<0.001	(↑) ns.	
	Crawler	<0.001	(↓) ns.	
	Burrower (epibenthic)	<0.05	(↑) ns.	
	Interstitial (endobenthic)	<0.05	(↑) ns.	
Food types	Fine sediment + microorganisms	<0.05	(↑) ns.	
	Living microphytes	<0.01	(↓) ns.	
	Living microinvertebrates	<0.01	(↓) ns.	
Feeding habits	Deposit feeder	<0.001	(↑) ns.	(↑) b
	Shredder	ns.	ns.	(↓) b
	Scraper	<0.05	(↓) ns.	(↓) b

was mainly attributable to only a few downstream CdS sites (Maella, Vallderoures and Nonasp), which did not show the expected terrestrial and endophytic clutches reproduction, eggs as a form of resistance or semivoltine cycles. This absence at these sites produced a marginal change in the slope of the relationship between the relative utilisation of each trait category in the up- and downstream sites in autumn 2015 and spring 2016 (Fig. 2.3b; SMA slope, $p < 0.01$ and $p < 0.01$, respectively). Changes in the Maella, Vallderoures and Nonasp sites were not so much related to the direct impact of effluents but to the massive growth of *Cladophora glomerata* mats in the downstream sites. SdS upstream sites showed differences in functional responses between dry and wet periods (Fig. 2.3c; SMA slope, $p < 0.01$). The temporal dispersion of functional traits was lower in the SdS downstream sites than in

their corresponding upstream sites (Fig. 2.3c and 2.3d; SMA slope, $p = 0.683$). The SdS had different functional responses in the three sampling campaigns (Fig. 2.3d), and the difference between upstream and downstream sites was more pronounced in the dry periods (summer and autumn; SMA slope, $p < 0.05$ and $p < 0.001$ respectively) when the relative utilisation of 40 trait categories significantly changed (Table S2.8). No differences between upstream and downstream sites in SdS were observed in functional traits utilisation in spring (Fig. 2.3d; SMA slope, $p = 0.108$).

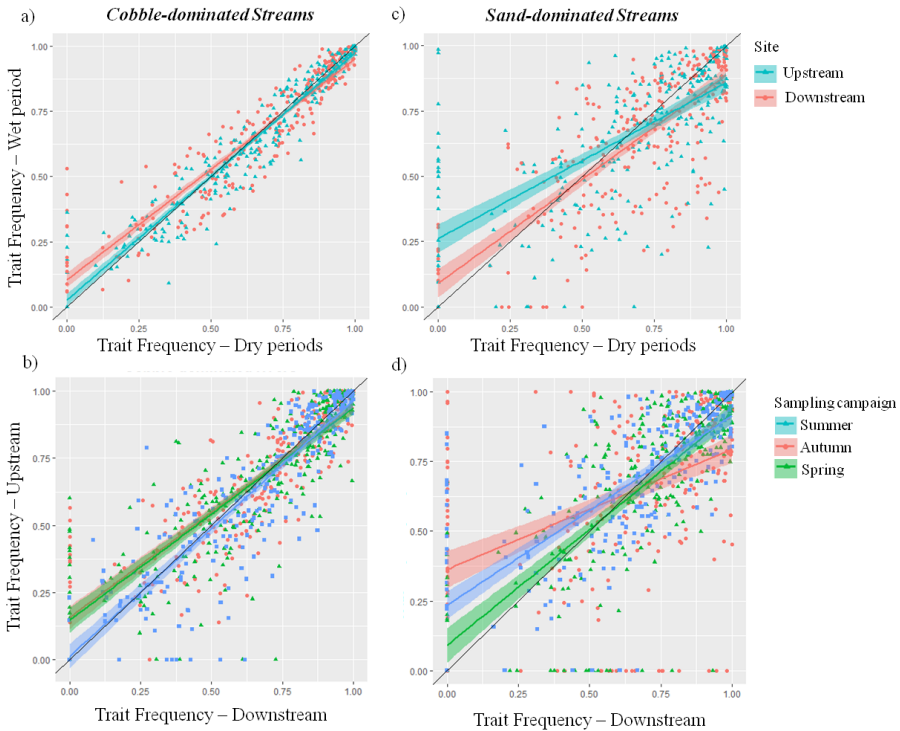


Fig. 2.3 Comparison of the relative utilization of each trait category among dry (summer and autumn) and wet (spring) periods in cobble-dominated streams (CdS) (a) and sand-dominated streams (SdS) (c), and between upstream and downstream reaches across the three periods in CdS (b) and SdS (d).

Discussion

Urban wastewater inputs caused a tenfold increase in nutrients, dissolved organic matter and pharmaceutical products appearing at the receiving sites. The chemical impact on the invertebrate assemblages was enhanced under the lowest dilution situations; taxonomic diversity and richness decreased, with particular effects on those species sensitive to pollution. Effects were also noticeable on the functional traits, both in the change in the relative utilization of trait categories and through a decrease of functional diversity. Furthermore, taxonomic and functional changes could not be fully understood without considering the stream size as well as the prevailing streambed substrata of the receiving sites. These differences engendered different responses to the urban wastewater pollution, increasing the dissimilarity of the invertebrate communities in the impacted sites.

Wastewater and hydrological variability effects

Urban wastewater effluents pollution caused a decrease in the macroinvertebrate and EPT richness as well as on overall diversity. Analogous results reported elsewhere (Arce et al., 2014; Ortiz and Puig, 2007) indicate that urban wastewater effluents perform as environmental filters for the pollution-tolerant taxa (Table 2.5). Co-inertia analysis showed that the shift in macroinvertebrate community composition towards Chironomidae and Oligochaeta dominance was proportional to the urban wastewater impacts (Burdon et al., 2016; Ortiz et al., 2005). Six functional trait categories, highly sensitive to nutrient and organic

matter pollution (Mondy et al., 2016), were altered in the impact sites (Table 2.6); these included the proportion of semivoltine individuals (less than one reproductive cycle per year) and eggs as a resistance formed in response to water intermittency. Aquatic organisms accumulate chemicals either as a result of predation (Ruhí et al., 2015a) or through their direct absorption from the water (Maulvault et al., 2018). The latter mechanism would favour organisms with lower surface-mass ratios and is likely to be associated to the increase in body size (Merckx et al., 2018). Small-sized organisms (those < 2mm) become less common since they present a higher surface-mass ratio and become more sensitive to pollution (e.g., Dolédec and Statzner, 2008). A lower occurrence of invertebrates breathing with gills could also be related to the high surface-mass ratio provided by these structures, making organisms more sensitive to pollution (e.g., Statzner and Bêche, 2010). The frequency of free-clutches reproduction increased in wastewater polluted sites. However, there is no direct mechanistic explanation and this frequency increase could be explained by the reduction of pollution sensitive reproduction structures, i.e., eggs (Mondy et al., 2016).

Streambed substrata and stream size were relevant factors driving the invertebrate responses. Cobble-dominated streams had more heterogeneous environments than sand-dominated streams and showed higher ability to support communities with greater taxonomic (Table 2.4) and functional diversity (Fig. 2.2c). Compacted and homogenous streambed substrata (as in sand-

dominated streams) can barely support highly diverse invertebrate assemblages (Duan et al., 2009).

In both stream types, the invertebrate community composition was strongly correlated to the chemical impact at the downstream sites (co-inertia analysis). However, this change did not translate into the invertebrate functional trait composition in sand-dominated streams. These latter streams differed from cobble-dominated streams in twelve trait categories (e.g., sand substrata enhanced the presence of burrowers). We suspect that the lower environmental heterogeneity in the sand-dominated streams accounted for the lower effects of wastewater effluents on the trait composition. Overall, these physical differences hosted specific pools of upstream invertebrate assemblages, which were the receptors of urban wastewater effluents. These different species pools might offer particular responses to pulsed perturbations (Clements et al., 2012) and, according to our results, also in the case of chronic impacts (such as wastewater inputs). This highlights the difficulty of making predictions about the wastewater impacts without considering the composition and traits of the receiving macroinvertebrate assemblages.

Even though wastewater discharges did not greatly altered the hydrological patterns of the receiving streams, they certainly transformed ephemeral or intermittent streams to permanent systems (Murdock et al., 2004). Similarly to Canobbio et al. (2009), we observed that more pollution-tolerant taxa, mainly Syrphidae and Culicidae, used downstream effluent sites as a permanent

water habitat. Differences also existed between the permanently flowing sampling locations, regarding their size and substrata type. Larger sampling locations (having both higher flow and cobble-dominated substrata) had a fairly constant biological trait occurrence through periods of different hydrology. In smaller sampling locations (lower water flow, sand-dominated; Fig. 2.3; Bêche et al., 2006), functional trait utilisation changed between dry and wet periods in the upstream sites. However, their downstream impacted sites had low dilution ability, causing stable, highly polluted environments (Mandarić et al., 2018). This downstream stability favoured the presence of highly pollution-tolerant taxa, leading to a more pronounced functional trait homogenisation between dry and wet periods (Fig. 2.2d).

Wastewater impact could increase macroinvertebrate community beta-diversity

Wastewater inputs did not cause the homogenisation of community composition among downstream sites, as we hypothesised. The dissimilarity (beta diversity) in the cobble-dominated streams was higher among the downstream sites than in their corresponding upstream sites, but this difference nearly disappeared in the sand-dominated streams. Human impacts might increase or decrease the beta diversity of the receiving communities, but the response patterns may differ according to the considered trophic level, scale, and stressor persistence (Socolar et al., 2016). The beta-diversity increase we recorded in the larger sampling locations could be related to several possible

causes: i) an increase of the environmental heterogeneity, produced by the wastewater input in sampling locations with high flow variability; testing this hypothesis further would require detailed measures of habitat availability and heterogeneity (Hawkins et al., 2015), not performed in our study; ii) an increase in stream productivity, promoted by wastewater effluents and favouring opportunistic responses on the biota which could be translated into a higher beta diversity (Hawkins et al., 2015); however, invertebrate richness was reduced, and the described beta-diversity increase is usually associated with an increase in the number of species (Chase, 2010), and iii) environmental differences (e.g. differences between sand- and cobble-dominated streams and periods), which could promote particular upstream species pools and could therefore contribute to the differences among downstream sites (Burdon et al., 2016). In our case, the taxa that contributed to the similarity among upstream sites were those that accounted for the difference between up and downstream sites (Table 2.5). Wastewater effluents reduced the common species and triggered the particular response of the downstream communities.

Concluding remarks

Urban wastewater effluents affect invertebrate assemblages in a complex manner, profoundly changing the composition and structure of the local communities. Different responses were also dependent on the local community pool, which depended on the stream characteristics (streambed structure and size of the system) and hydrology (discharge and flow variability). A clear implication

of our findings is that wastewater management policies should pay greater attention to the receiving ecosystems (i.e., hydrology and stream characteristics), and not just to the concentration of pollutants at the wastewater effluent sites.



CHAPTER 3

The silent predator: effects of urban wastewater on community size structure and energy transfer efficiency through river food webs.

Mor, J.R., I. Muñoz, S. Sabater, LL. Zamora and A. Ruhi. IN PREP.

GENERAL DISCUSSION

A photograph of a rocky stream flowing through a valley. The stream is shallow and clear, with numerous light-colored rocks visible in the foreground and middle ground. The banks are covered with green grasses and shrubs. In the background, there are green hillsides with sparse vegetation under a clear sky. The overall scene is a natural, outdoor setting.

This thesis analyses the effects of water scarcity -and the associated dam alterations and urban wastewater pollution- on river food webs and invertebrate communities. The main results highlight the importance of stream hydrology to determine ecosystem composition, stability and functioning (Chapter 1 and 2). Flow regimes control substrata composition, channel morphology and size, which together influence habitat characteristics and consequently the abundance, distribution, and diversity of organisms (Power et al., 1995; Nilsson and Svedmark, 2002). On one hand, dam induced hydrological stability and changed detritus-based to algal-based food webs, producing a change on the downstream community composition and widening and lengthening the food webs (Chapter 1). On the other hand, different flow regimes determined different communities that respond differently to urban wastewater pollution (Chapter 2). In these cases, river discharge also determined the dilution capacity of a system to pollutants (e.g., Rice and Westerhoff, 2017), and consequently the pollutant concentrations received by the biological communities (Chapter 2). These results strengthen the idea that hydrological patterns might be considered a “master variable” that largely determine the riverine communities as well as the intensity of impacts they receive (Death and Winterbourn, 1995; Bunn and Arthington, 2002). Hydrological alteration and wastewater pollution affect the relative importance of allochthonous inputs and autochthonous production at the base of the food webs (Chapter 1 and 3). Additionally, create physical and chemical constraints to trophic interactions, community body size,

and energy pathways, decreasing food webs' temporal stability (Chapter 1 and 3).

In this general discussion the observations and experiences noted in the different chapters are combined to develop generalized statements.

Anthropogenic impacts lead to stream community's homogenization? The community contribution

Local biodiversity (i.e., species richness and α -diversity) is decreasing elsewhere due to anthropogenic habitat degradation (Loreau, 2010). Less clear is how much the anthropogenic impacts affect diversity from a regional point of view (γ and β -diversities). It is assumed that communities confronted by a same impact will tend to homogenization (i.e., achieving a reduction of the β -diversity), as a cause of the reduction of the environmental heterogeneity (e.g., Maloney et al., 2011; Mcgoff et al., 2013; Passy and Blanchet, 2007). Still, some studies have found an increase of β -diversity, suggesting that different responses could exist to a same impact, this being at spatial, temporal and trophic guild perspectives (Socolar et al., 2016). This has motivated a new research focus analysing why these differences may occur. Either acting separately or jointly, changes in environmental heterogeneity, species differences in stressor tolerance, changes in ecosystem productivity or changes in connectivity between sites, have been proposed as mechanisms to explain differences of β -diversity between human-impacted sites (Hawkins et al., 2015).

This thesis proposes that different communities can respond differently to the same stressor. This would perform as a mechanism accounting for the increase in differences among impacted sites (β -diversity). The spatial perspective, analysed in Chapter 2, shows that urban wastewater pollution increases regional differences (β -diversity) between impacted communities. This result did not confirm our hypothesis, but showed that different habitats (e.g., high heterogeneous habitats *versus* less diverse habitats) had different community composition and different responses to human impacts. At the same time, these communities received different degree of impact, and were submitted to a larger range of environmental conditions than non-polluted communities. These two aspects might lead to higher differences in community composition among impacted sites. However, we also observed that wastewater pollution produced a homogenization of the diatom community (Tornés et al., 2018). This different response on β -diversity between biological communities that coexist at the same time and place can occur if we look at different biological groups and could add the ability to colonize after perturbations (e.g., floods) and the composition of the metacommunity pool of species, as new factors modulating the β -diversity response (but see Heino et al., 2015).

The temporal changes in the invertebrate community when receives urban wastewater pollution, indicates that those inhabiting streams with lower habitat heterogeneity present a stable functional composition when exposed to different hydrological

conditions. This contrasts with the temporal trait variability occurring between different hydrological periods at the upstream, non-polluted sites (Chapter 2). I therefore conclude that the two types of anthropogenic impacts (regulation and pollution) cause a reduction of the temporal stability of food webs, even taking into account the ‘snapshot’ nature of my study. Dams reduce the hydrological variability, facilitating the terrestriality of the river streambed (Chapter 1), and under these conditions the diet of top-predators are subsidized by terrestrial invertebrates, reducing their pressure on the fluvial food web, and allowing an increase of the trophic redundancy and food-chain-length. However, when the terrestrial invertebrate input is interrupted (i.e. mediated by the seasonality), top-predators shift their diet towards aquatic preys, thereby producing a top-down effect with a temporal change of the communities (increase of temporal β -diversity). In a similar vein, food webs from urban wastewater polluted sites (Chapter 3) presented a reduction of the energy efficiency transfer along the food web, as well as narrower predators’ diet breadth (i.e., smaller diet niche) and a larger predator-prey mass ratio. These characteristics suggest that these food webs might be less resilient to other perturbations e.g., floods (Plank and Law, 2011), and be its communities subject to post-perturbation colonisations that might increase the temporal β -diversity (e.g., Cauvy-Fraunié et al., 2015).

Even taking into account the large range of scenarios that we could obtain after altering the community composition when

mediated by anthropogenic impacts, community homogenization based on structural (taxonomical) or functional composition cannot be discarded. The increase of invasive species leads to taxonomic and functional homogenisation worldwide (e.g., Comte and Olden, 2017; Olden et al., 2018, 2016). In this thesis, the reduction of predator's richness and abundance, together with an increment on the number of trophic end-points (i.e., energy not flowing to higher trophic levels; Chapter 3), could benefit the entrance of some invasive species (Shea and Chesson, 2002). Here, the observed β -diversity increase could be reversed, and the fragility of these perturbed systems can lead to a homogenization of the freshwater communities.

Anthropogenic impacts change ecosystem boundaries

The anthropogenic impacts can constrain species mobility by physical (i.e., dam, Chapter 1) and pollution obstacles (i.e., urban wastewater pollution, Chapter 2 and 3). Organisms and organic matter movement across habitat boundaries from other ecosystems are key to maintain community structure and predators density (Huxel and McCann, 1998; Polis and Hurd, 1995). The dam presence could physically isolate upstream communities limited to species with capacity to overcome the dam obstacle (mainly by terrestrial or aerial dispersion) or to those contributed from neighbouring freshwater ecosystems. Pollution impact also acts as an environmental filter selecting pollutant tolerant species (Chapter 2) and reducing the contribution of larger taxa that

mainly are predators (Chapter 3). Larger species usually have higher habitat requirements than smaller ones (Tamburello et al., 2015), combining local energy sources with others that come from ‘neighbouring’ food webs (i.e., stream communities) or even other ecosystems (e.g., Nakano et al., 1999a; Polis and Hurd, 1995). In that case, reducing larger sized species changes community structure from being inverse-biomass pyramid in the non-polluted sites (i.e., predators have a higher biomass density than potential preys) to being a lower density of predators than preys on polluted sites (Chapter 3). Wastewater pollution reduces the movement range of predators, and jointly with the reduction on the energy efficiency transfer and larger predator-prey mass ratios (Chapter 3), might also reduce community composition stability over time (Plank and Law, 2011). These findings, highlights that energy exchanges between stream food webs (i.e., species mobility) are key to maintain the highest trophic levels (Polis and Hurd, 1995). The two studied anthropogenic impacts not only affect the species mobility but also the energy flow, mostly in the species of the highest trophic levels.

Learning from the thesis: Implications for future research

All studies have a proper development framework, and this is not the exception. Here we discuss the main methodological constraints associated to the current framework, and which may have affected the results of the thesis:

Replication of study sites

Dam longitudinal impacts on stream food web (Chapter 1) consisted on a single sampling, but the structure and dynamics of stream food webs are highly seasonal (Power et al., 2008). Thus, a single sampling in a given season represents a fraction of the actual trophic dynamics, even when the sampling was performed in the most active period (spring). The quality of food webs data is determined by the documentation of species richness and abundance and the interactions strengths between all species within the food web (Rooney et al., 2008). In the trade-off between having subsequent samplings or having a highly-resolved food web, we chose the latter option. While several studies have described seasonal variation in Mediterranean-climate river assemblages (e.g. Acuña et al. 2005; Bonada et al. 2006), none had described so far the biotic interactions at the species level. The choice of a longitudinal sampling design, is common in studying the effects of dams (e.g. Cross et al., 2013; Katano et al., 2009; Voelz and Ward, 1991), but limits the strength of the evidence. That is, the ability to extrapolate beyond our study system is limited, but was sufficient to capture the dam-induced increase in hydrologic stability and the responses of the food web. Other approaches like mesocosm experimentation could amplify replication and manipulation of the environmental regime, but would not capture the complexity and history of the community, nor would they represent the indirect perturbations caused by the dam-induced hydrologic stability (e.g., terrestrialization). Thus, despite the high cost of food web analysis, further research along

these lines could provide a wider point of view of the effects of dams on Mediterranean stream food webs.

Control sites

Establishing control sites to understand the anthropogenic impacts can be complex in highly altered regions such as the Mediterranean basin (Rundel et al., 1998). In this thesis the terms “upstream” and “downstream” were used to refer to the non-impacted and impacted sites. However, in which measure upstream sites could be considered a control site? In the framework of systems having a long history of human influences, the two alterations (i.e., dams and urban wastewater pollution) could perform as obstacles affecting upstream communities (Chapters 1 and 3). Additionally, some rivers could be impacted by agriculture or other activities having high concentrations of nutrients or other pollutants (Chapter 2). However, Mediterranean streams present a high variability in their community composition (e.g., Chapter 2), and determining the changes produced by the impacts using an upstream site as control may actually be better in capturing the localized impact effects (while controlling other nuances such as variation in landscape and river basin characteristics).

Additionally, a second question could appear: how do we know that observed differences were produced by the impacts and there is no a natural change associated to the distance between the sites? On Chapters 2 and 3, the distance between up and downstream sites was determined by the stream size (the

downstream sites had to achieve a complete mixture of effluents and stream waters, and consequently larger rivers needed a greater distance than smaller streams). So forth, upstream and downstream sites were situated between 200 m and 1300 m from each other. Considering both the previous knowledge about these rivers and the altitude of sites, which was the same in the upstream and downstream, the only difference was the discharge of urban wastewater effluents being received by the downstream sites. However, the dam effects on Chapter 1 were studied along a section that ranged from 12.3 Km upstream to 14.2 km downstream the dam, and even there was not large changes on altitude and land uses, the community composition could easily change along a section of 26 km. Nevertheless, Mediterranean streams present such a high variability in their community composition (e.g. Chapter 2), that a longitudinal design is the best option to capture the localized effects of the dam. Longitudinal studies are common to investigate the effects of dams (e.g. Voelz & Ward 1991; Katano et al. 2009; Cross et al. 2013). Although longitudinal studies limit the strength of the evidence (or the ability to extrapolate beyond our study system), our study captured well the dam-induced increase in hydrologic stability, and the responses of the food web (please see figure 1.4).

Identification of direct and indirect impacts

Anthropogenic impacts could act as direct stressors on biological communities (Chapter 2). In these cases an action-response link could be established. In other cases, indirect impacts

could modify the response of a community. For example, nutrients within the wastewater inputs can increase primary production and increase the biomass of first consumers (Chapter 3); and, water stability imposed by a dam can increase the entrance of terrestrial vegetation, reducing the river width and favouring the predation of terrestrial insects by fishes consequently reducing its pressure on the aquatic food web (Chapter 1). Some of these indirect effects have brought out the close relationship between aquatic and terrestrial ecosystems. Determining the study of the ecosystem boundaries can be problematic when inputs and outputs of the system operate at different spatial and temporal scales (e.g., if stream primary production is affected by river width and growth of the riverine forest on the river bed linked to the reduction of annual flow variability; Chapter 1) and particularly when there is not a strong association between community composition, resource supply and physical boundaries (Post et al., 2007). Thus, studying food webs we cannot only consider physical stream boundaries but also energy fluxes even if they come from other stream reaches (Chapter 3) or ecosystems (Chapter 1).

Information about functional traits

To analyse the functional response of the invertebrate community to wastewater pollution, trait categories were obtained from public databases for European macroinvertebrate taxa (mainly at the genus level; Tachet et al., 2010), with some adaptations for the Mediterranean region (Bonada et al., 2007; Bonada and Dolédec, 2011). However, species could change its

behaviour according to their adaptability to the environmental conditions. For example, *Ancylus fluviatilis* (Gastropoda, Planorbidae) and Orthocladinae (Diptera, Chironomidae) had a detritivorous diet in upstream intermittent sites and an herbivorous diet in the dam downstream sites (Chapter 1). In order to consider possible variability in species taxon affinity, each trait category was quantified using fuzzy coding and further used as a profile (see Chevenet et al., 1994). Fuzzy code enables us to consider the species variability, but additional research is needed to complete existing databases (Statzner and Bêche, 2010). This could be achieved through field and laboratory experiments, but the information gap in some taxonomic groups such as dipterans and oligochaetes is too large (Statzner and Bêche, 2010). In addition, these groups are generally not well identified due to difficult taxonomical determination. Because some species could shift their traits in some circumstances, even if the database was completed in the end, we would not be able to relate all direct effects and we must accordingly consider this analysis as a first approach.

Future directions

Finally, further research is necessary to successfully link food web studies and ecosystem functioning. Nowadays, food web analyses are trendy again to evaluate the ecosystem and the impacts it receives (e.g., Harvey et al., 2017). Although the food web perspective is considered a key point of view for a holistic analysis of the whole community and species interactions (Woodward and Hildrew, 2002a), there is no link among its metrics and the

necessary management actions or the repercussions that a metrics change could have on the ecosystem.

Regarding the effects on food webs studied in this thesis, more research is needed in both dam and wastewater pollution. In particular, there is little literature studying these impacts in the Mediterranean regions, and species adaptability and functional loss by the increase of anthropogenic pressures could be solved by means of additional food web studies. Additionally, we hypothesized in Chapter 3 that pollutant wastewater effects could be considered as “new species” on the food web structure that affect trophic interactions. Experiments using mesocosms could help to understand the effects that pollutants can provoke on food web structure and on the species interactions.

Conclusions

Chapter 1: *Dam regulation and riverine food-web structure in a Mediterranean river*

1. Flow regulation by dams can alter food-web structure in intermittent rivers, not only via changes in community composition but also via changes in the relative importance of autochthonous production *vs.* allochthonous inputs.
2. Flow regulation increase basal autochthony. There is a shift from a detritus-based to algal-based food webs widening and lengthening food webs.
3. The discontinuity imposed by dams may present cumulative effects on food webs, and impacts of flow regulation by dams may persist even if the physical template is locally restored.

Chapter 2: *Invertebrate community responses to urban wastewater effluent pollution under different hydro-morphological conditions*

1. Wastewater pollution drives taxonomic and functional changes in benthic communities, and these are enhanced under low dilution situations.
2. Wastewater pollution act as environmental filter selecting pollutant tolerant taxa.

3. Wastewater pollution effects differ according to the receiving community. Stream size, the prevailing streambed substrata and the river discharge produce different responses.

Chapter 3: *The silent predator: effects of urban wastewater on community size structure and energy transfer efficiency through river food webs*

1. Wastewater pollution simplifies the food web, affecting all consumers and the predator assemblage and, in particular, reducing the trophic interactions.
2. Wastewater pollution reduces the energy efficiency transfer on impacted communities.
3. Wastewater pollution mainly affects larger species, reducing predator diet breadth.
4. Wastewater pollution reduces the recovery capacity of the impacted communities to other perturbations.

General Conclusions

1. Anthropogenic impacts might increase or reduce β -diversity suggesting that different communities can respond differently to the same stressor, explaining different responses observed from spatial, temporal and trophic guild perspectives.
2. Anthropogenic impacts can constrain species mobility by physical and pollution and changing community structure and temporal stability.

3. The magnitude of the effects showed in this thesis should be considered in a better planning of new dams and water regulation action. In addition, wastewater management policies should consider the characteristics of the receiving ecosystems (i.e. hydrology and stream characteristics), and not just the pollutant concentrations at the effluents.

References

Abril, M., Muñoz, I., Casas-Ruiz, J.P., Gómez-Gener, L., Barceló, M., Oliva, F., Menéndez, M., 2015. Effects of water flow regulation on ecosystem functioning in a Mediterranean river network assessed by wood decomposition. *Sci. Total Environ.* 517, 57–65. doi:10.1016/j.scitotenv.2015.02.015

Acuña, V., Muñoz, I., Giorgi, A., Omella, M., Sabater, F., Sabater, S., 2005. Drought and postdrought recovery cycles in an intermittent Mediterranean stream: structural and functional aspects. *J. North Am. Benthol. Soc.* 24, 919–933. doi:10.1899/04-078.1

Ahrens, W.H., Cox, D.J., Budhwar, G., 1990. Use of the Arcsine and Square Root Transformations for Subjectively Determined Percentage Data. *Weed Sci.* 38, 452–458. doi:10.1017/S0043174500056824

Allan, J.D., Castillo, M., 2007. *Stream Ecology*.

Arce, E., Archaimbault, V., Mondy, C.P., Usseglio-Polatera, P., 2014. Recovery dynamics in invertebrate communities following water-quality improvement: taxonomy- vs trait-based assessment. *Freshw. Sci.* 33, 1060–1073. doi:10.1086/678673

Arenas-sánchez, A., Rico, A., Vighi, M., 2016. Effects of water scarcity and chemical pollution in aquatic ecosystems : State of the art. *Sci. Total Environ.* 572, 390–403.

doi:10.1016/j.scitotenv.2016.07.211

Arim, M., Abades, S.R., Laufer, G., Loureiro, M., Marquet, P.A., 2010. Food web structure and body size: trophic position and resource acquisition. *Oikos* 119, 147–153. doi:10.1111/j.1600-0706.2009.17768.x

Aristi, I., Arroita, M., Larrañaga, A., Ponsatí, L., Sabater, S., von Schiller, D., Elozegi, A., Acuña, V., 2014. Flow regulation by dams affects ecosystem metabolism in Mediterranean rivers. *Freshw. Biol.* 59, 1816–1829.

Aristi, I., von Schiller, D., Arroita, M., Barceló, D., Ponsatí, L., García-Galán, M.J., Sabater, S., Elozegi, A., Acuña, V., 2015. Mixed effects of effluents from a wastewater treatment plant on river ecosystem metabolism: Subsidy or stress? *Freshw. Biol.* 60, 1398–1410. doi:10.1111/fwb.12576

Artigas, J., Romaní, A.M., Gaudes, A., Muñoz, I., Sabater, S., 2009. Organic matter availability structures microbial biomass and activity in a Mediterranean stream. *Freshw. Biol.* 54, 2025–2036.

Baird, D., Ulanowicz, R.E., 1989. The Seasonal Dynamics of The Chesapeake Bay Ecosystem. *Ecol. Monogr.* 59, 329. doi:10.2307/1943071

Barber, L.B., Brown, G.K., Nettesheim, T.G., Murphy, E.W., Bartell, S.E., Schoenfuss, H.L., 2011. Effects of biologically-active chemical mixtures on fish in a wastewater-impacted urban stream. *Sci. Total Environ.* 409, 4720–4728.

doi:10.1016/j.scitotenv.2011.06.039

Barton, K., 2018. MuMIn: Multi-Model Inference. R package version 1.40.4.

Baselga, A., 2017. Partitioning abundance-based multiple-site dissimilarity into components: balanced variation in abundance and abundance gradients. *Methods Ecol. Evol.* 8, 799–808. doi:10.1111/2041-210X.12693

Baselga, A., 2010. Partitioning the turnover and nestedness components of beta diversity. *Glob. Ecol. Biogeogr.* 19, 134–143. doi:10.1111/j.1466-8238.2009.00490.x

Baselga, A., Orme, D., Vileger, S., Bortoli, J. De, Leprieur, F., 2017. betapart: Partitioning Beta Diversity into Turnover and Nestedness Components. R package version 1.4-1.

Batalla, R.J., Gómez, C.M., Kondolf, G.M., 2004. Reservoir-induced hydrological changes in the Ebro River basin (NE Spain). *J. Hydrol.* 290, 117–136. doi:10.1016/j.jhydrol.2003.12.002

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* 67, 1–48. doi:doi:10.18637/jss.v067.i01

Bêche, L.A., McElravy, E.P., Resh, V.H., 2006. Long-term seasonal variation in the biological traits of benthic-macroinvertebrates in two Mediterranean-climate streams in California, U.S.A. *Freshw. Biol.* 51, 56–75. doi:10.1111/j.1365-

2427.2005.01473.x

Belmar, O., Bruno, D., Martínez-Capel, F., Barquín, J., Velasco, J., 2013. Effects of flow regime alteration on fluvial habitats and riparian quality in a semiarid Mediterranean basin. *Ecol. Indic.* 30, 52–64. doi:10.1016/j.ecolind.2013.01.042

Benke, A.C., Huryn, A.D., Smock, L.A., Wallace, J.B., 1999. Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *J. North Am. Benthol. Soc.* 308–343.

Blanchard, J.L., Jennings, S., Law, R., Castle, M.D., McCloghrie, P., Rochet, M., Benoît, E., 2009. How does abundance scale with body size in coupled size-structured food webs? *J. Anim. Ecol.* 78, 270–280. doi:10.1111/j.1365-2656.2008.01466.x

Boix, D., García-Berthou, E., Gascón, S., Benejam, L., Tornés, E., Sala, J., Benito, J., Munné, A., Sola, C., Sabater, S., 2010. Response of community structure to sustained drought in Mediterranean rivers. *J. Hydrol.* 383, 135–146.

Bonada, N., Dallas, H., Rieradevall, M., Prat, N., Day, J., 2006. A comparison of rapid bioassessment protocols used in 2 regions with Mediterranean climates, the Iberian Peninsula and South Africa. *J. North Am. Benthol. Soc.* 25, 487–500. doi:10.1899/0887-3593(2006)25[487:ACORBP]2.0.CO;2

Bonada, N., Dolédec, S., 2011. Do mediterranean genera not included in Tachet et al. 2002 have mediterranean trait

characteristics? *Limnetica* 30, 129–142.

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. *Glob. Chang. Biol.* 13, 1658–1671. doi:10.1111/j.1365-2486.2007.01375.x

Bonada, N., Resh, V.H., 2013. Mediterranean-climate streams and rivers: geographically separated but ecologically comparable freshwater systems. *Hydrobiologia* 719, 1–29.

Bonada, N., Rieradevall, M., Prat, N., Resh, V.H., 2006. Benthic macroinvertebrate assemblages and macrohabitat connectivity in Mediterranean-climate streams of northern California. *J. North Am. Benthol. Soc.* 25, 32–43.

Bowen, S.H., 1987. Composition and nutritional value of detritus., in: Moriarty, D.J.W., Pullin, R.S.V. (Eds.), *Detritus and Microbial Ecology in Aquaculture*. ICLARM Conference Proceedings 14. International Center for Living Aquatic Resources Management, Manila, Philippines, pp. 192–216. doi:10.2307/1467571

Bretz, F., Hothorn, T., Westfall, P., 2010. *Multiple Comparisons Using R*. Chapman and Hall/CRC. doi:10.1201/9781420010909

Brittain, J.E., Saltveit, S.J., 1989. A review of the effect of river regulation on mayflies (Ephemeroptera). *Regul. Rivers Res. Manag.* 3, 191–204. doi:10.1002/rrr.3450030119

References

Brose, U., Blanchard, J.L., Eklöf, A., Galiana, N., Hartvig, M., R. Hirt, M., Kalinkat, G., Nordström, M.C., O’Gorman, E.J., Rall, B.C., Schneider, F.D., Thébault, E., Jacob, U., 2016. Predicting the consequences of species loss using size-structured biodiversity approaches. *Biol. Rev.* 49. doi:10.1111/brv.12250

Brose, U., Jonsson, T., Berlow, E.L., Warren, P., Banasek-Richter, C., Bersier, L.-F.L., Blanchard, J.L., Brey, T., Carpenter, S.R., Blandenier, M.-F.C., Cushing, L., Dawah, H.A., Dell, T., Edwards, F., Harper-Smith, S., Jacob, U., Ledger, M.E., Martinez, N.D., Memmott, J., Mintenbeck, K., Pinnegar, J.K., Rall, B.R.C., Rayner, T.S., Reuman, D.C., Ruess, L., Ulrich, W., Williams, R.J., Woodward, G., Cohen, J.E., 2006. Consumer-resource body-size relationship in natural food webs. *Ecology* 87, 2411–2417.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Toward a metabolic theory of ecology. *Ecology* 85, 1771–1789.

Bunn, S.E., Arthington, A.H., 2002. Basic Principles and Ecological Consequences of Altered Flow Regimes for Aquatic Biodiversity. *Environ. Manage.* 30, 492–507. doi:10.1007/s00267-002-2737-0

Burdon, F.J., Reyes, M., Alder, A.C., Joss, A., Ort, C., Räsänen, K., Jokela, J., Eggen, R.I.L., Stamm, C., 2016. Environmental context and magnitude of disturbance influence trait-mediated community responses to wastewater in streams. *Ecol. Evol.* 6, 3923–3939. doi:10.1002/ece3.2165

Burgherr, P., Meyer, E.I., 1997. Regression analysis of linear body dimensions vs. dry mass in stream macroinvertebrates. *Arch. für Hydrobiol.* 139, 101–112.

Cabana, G., Rasmussen, J.B., 1996. Comparison of aquatic food chains using nitrogen isotopes. *Proc. Natl. Acad. Sci.* 93, 10844–10847.

Calizza, E., Costantini, M.L., Rossi, L., 2015. Effect of multiple disturbances on food web vulnerability to biodiversity loss in detritus-based systems. *Ecosphere* 6, 1–20. doi:10.1890/ES14

Canobbio, S., Mezzanotte, V., Sanfilippo, U., Benvenuto, F., 2009. Effect of Multiple Stressors on Water Quality and Macroinvertebrate Assemblages in an Effluent-Dominated Stream. *WATER AIR SOIL Pollut.* 198, 359–371. doi:10.1007/s11270-008-9851-4

Carey, R.O., Migliaccio, K.W., 2009. Contribution of wastewater treatment plant effluents to nutrient dynamics in aquatic systems: a review. *Environ. Manage.* 44, 205–17. doi:10.1007/s00267-009-9309-5

Carlisle, D.M., Wolock, D.M., Meador, M.R., 2011. Alteration of streamflow magnitudes and potential ecological consequences: A multiregional assessment. *Front. Ecol. Environ.* 9, 264–270. doi:10.1890/100053

Cauvy-Fraunié, S., Andino, P., Espinosa, R., Jacobsen, D., Dangles, O., 2015. Temporal scaling of high flow effects on

References

benthic fauna: Insights from equatorial glacier-fed streams. *Limnol. Oceanogr.* 60, 1836–1847. doi:10.1002/lno.10137

Charvet, S., Kosmala, A., Statzner, B., 1998. Biomonitoring through biological traits of benthic macroinvertebrates: perspectives for a general tool in stream management. *Fundam. Appl. Limnol.* 142, 415–432. doi:10.1127/archiv-hydrobiol/142/1998/415

Chase, J.M., 2010. Stochastic community assembly causes higher biodiversity in more productive environments. *Science* (80-.). 328, 1388–1391. doi:10.1126/science.1187820

CHE, 2013. Propuesta de proyecto de plan hidrológico de la cuenca del Ebro. Inventario de presiones.

Chessel, D., Dufour, A.B., Thioulouse, J., 2004. The ade4 package-I- One-table methods. *R News* 4, 5–10.

Chevenne, F., Dolédec, S., Chessel, D., 1994. A fuzzy coding approach for the analysis of long-term ecological data. *Freshw. Biol.* 31, 295–309. doi:10.1111/j.1365-2427.1994.tb01742.x

Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. *Austral Ecol.* 18, 117–143. doi:10.1111/j.1442-9993.1993.tb00438.x

Clements, W.H., Hickey, C.W., Kidd, K.A., 2012. How Do Aquatic Communities Respond to Contaminants? It Depends on the Ecological Context. *Environ. Toxicol. Chem.* 31, 1932–1940.

doi:10.1002/etc.1937

Comte, L., Olden, J.D., 2017. Climatic vulnerability of the world's freshwater and marine fishes. *Nat. Clim. Chang.* 7, 718–722. doi:10.1038/nclimate3382

Corcoll, N., Casellas, M., Huerta, B., Guasch, H., Acuña, V., Rodríguez-Mozaz, S., Serra-Compte, A., Barceló, D., Sabater, S., 2015. Effects of flow intermittency and pharmaceutical exposure on the structure and metabolism of stream biofilms. *Sci. Total Environ.* 503–504, 159–170. doi:10.1016/j.scitotenv.2014.06.093

Coumou, D., Rahmstorf, S., 2012. A decade of weather extremes. *Nat. Clim. Chang.* 2, 491–496.

Cross, W.F., Baxter, C. V., Rosi-Marshall, E.J., Hall, R.O., Kennedy, T.A., Donner, K.C., Kelly, H.A.W., Seegert, S.E.Z., Behn, K.E., Yard, M.D., 2013. Food-web dynamics in a large river discontinuum. *Ecol. Monogr.* 83, 311–337. doi:10.1890/12-1727.1

Cucherousset, J., Villéger, S., 2015. Quantifying the multiple facets of isotopic diversity : New metrics for stable isotope ecology. *Ecol. Indic.* 56, 152–160. doi:10.1016/j.ecolind.2015.03.032

Dallman, P.R., 1998. *Plant Life in the World's Mediterranean Climates*. Berkeley: Calif. Native Plant Soc., Univ. Calif. P.

De Castro-Català, N., Muñoz, I., Riera, J.L., Ford, A.T., 2017. Evidence of low dose effects of the antidepressant fluoxetine and the fungicide prochloraz on the behavior of the keystone

References

- freshwater invertebrate *Gammarus pulex*. *Environ. Pollut.* 231, 406–414. doi:10.1016/j.envpol.2017.07.088
- Death, R.G., Winterbourn, M.J., 1995. Diversity Patterns in Stream Benthic Invertebrate Communities: The Influence of Habitat Stability. *Ecology* 76, 1446. doi:10.2307/1938147
- Dolédec, S., Chessel, D., 1994. Co-inertia analysis: an alternative method for studying species-environment relationships. *Freshw. Biol.* 31, 277–294. doi:10.1111/j.1365-2427.1994.tb01741.x
- Dolédec, S., Statzner, B., 2008. Invertebrate traits for the biomonitoring of large European rivers: An assessment of specific types of human impact. *Freshw. Biol.* 53, 617–634. doi:10.1111/j.1365-2427.2007.01924.x
- Dolédec, S., Statzner, B., Bournard, M., 1999. Species traits for future biomonitoring across ecoregions: patterns along a human-impacted river. *Freshw. Biol.* 42, 737–758.
- Döll, P., Fiedler, K., Zhang, J., 2009. Global-scale analysis of river flow alterations due to water withdrawals and reservoirs. *Hydrol. Earth Syst. Sci.* 13, 2413–2432. doi:10.5194/hess-13-2413-2009
- Dossena, M., Yvon-Durocher, G., Grey, J., Montoya, J.M., Perkins, D.M., Trimmer, M., Woodward, G., 2012. Warming alters community size structure and ecosystem functioning. *Proc. R. Soc. B Biol. Sci.* 279, 3011–3019. doi:10.1098/rspb.2012.0394
- Dray, S., Dufour, A.B., 2007. The ade4 package: implementing the

- duality diagram for ecologists. *J. Stat. Softw.* 22, 1–20.
- Dray, S., Dufour, A.B., Chessel, D., 2007. The ade4 package-II: Two-table and K-table methods. *R News* 7, 47–52.
- Duan, X., Wang, Z., Xu, M., Zhang, K., 2009. Effect of streambed sediment on benthic ecology. *Int. J. Sediment Res.* 24, 325–338. doi:10.1016/S1001-6279(10)60007-8
- Dynesius, M., Nilsson, C., 1994. Fragmentation and flow regulation of river systems in the northern third of the world. *Science* 266, 753–762. doi:10.1126/science.266.5186.753
- Edwards, A.W.F., 1971. Distances between Populations on the Basis of Gene Frequencies. *Biometrics* 27, 873. doi:10.2307/2528824
- EEA, 2018. European Waters Assessment of Status and Pressures.
- Francés, F., Vélez, J.I., Vélez, J.J., 2007. Split-parameter structure for the automatic calibration of distributed hydrological models. *J. Hydrol.* 332, 226–240. doi:10.1016/j.jhydrol.2006.06.032
- Frost, P.C., Stelzer, R.S., Lamberti, G.A., Elser, J.J., 2002. Ecological stoichiometry of trophic interactions in the benthos: Understanding the role of C:N:P ratios in lentic and lotic habitats. *J. North Am. Benthol. Soc.* 21, 515–528.
- Gasith, A., Resh, V.H., 1999a. Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. *Annu. Rev. Ecol. Syst.* 30, 51–81.

doi:10.1146/annurev.ecolsys.30.1.51

Gasith, A., Resh, V.H., 1999b. Streams in Mediterranean climate regions: Abiotic influences and biotic responses to predictable seasonal events. *Annu. Rev. Ecol. Syst.* 51–81.

Ginebreda, A., Muñoz, I., de Alda, M.L., Brix, R., López-Doval, J., Barceló, D., 2010. Environmental risk assessment of pharmaceuticals in rivers: Relationships between hazard indexes and aquatic macroinvertebrate diversity indexes in the Llobregat River (NE Spain). *Environ. Int.* 36, 153–162. doi:10.1016/j.envint.2009.10.003

Graça, M.A.S., Maltby, L., Calow, P., 1993. Importance of fungi in diet of *Gammarus pulex* and *Asellus aquaticus*. I. Feeding strategies. *Oecologia* 93, 139–144.

H Hairston Jr, N.G., Hairston Sr, N.G., 1993. Cause-effect relationships in energy flow, trophic structure, and interspecific interactions. *Am. Nat.* 142, 379–411.

Hall, R.O., Wallace, J.B., Eggert, S.L., 2000. Organic matter flow in stream food webs with reduced detrital resource base. *Ecology* 81, 3445–3463. doi:10.1890/0012-9658(2000)081[3445:OMFISF]2.0.CO;2

Harvey, E., Gounand, I., Ward, C.L., Altermatt, F., 2017. Bridging ecology and conservation: from ecological networks to ecosystem function. *J. Appl. Ecol.* 54, 371–379. doi:10.1111/1365-2664.12769

- Hawkins, C.P., Mykrä, H., Oksanen, J., Vander Laan, J.J., 2015. Environmental disturbance can increase beta diversity of stream macroinvertebrate assemblages. *Glob. Ecol. Biogeogr.* 24, 483–494. doi:10.1111/geb.12254
- Hecnar, S., McCloskey, R., 1997. The effects of predatory fish on amphibian species richness and distribution. *Biol. Conserv.*
- Heino, J., Melo, A.S., Bini, L.M., Altermatt, F., Al-Shami, S.A., Angeler, D.G., Bonada, N., Brand, C., Callisto, M., Cottenie, K., Dangles, O., Dudgeon, D., Encalada, A., Göthe, E., Grönroos, M., Hamada, N., Jacobsen, D., Landeiro, V.L., Ligeiro, R., Martins, R.T., Miserendino, M.L., Md Rawi, C.S., Rodrigues, M.E., Roque, F. de O., Sandin, L., Schmera, D., Sgarbi, L.F., Simaika, J.P., Siqueira, T., Thompson, R.M., Townsend, C.R., 2015. A comparative analysis reveals weak relationships between ecological factors and beta diversity of stream insect metacommunities at two spatial levels. *Ecol. Evol.* 5, 1235–1248. doi:10.1002/ece3.1439
- Hernando, M.D., Heath, E., Petrovic, M., Barceló, D., 2006. Trace-level determination of pharmaceutical residues by LC-MS / MS in natural and treated waters . A pilot-survey study. *Anal. Bioanal. Chem.* 385, 985–991. doi:10.1007/s00216-006-0394-5
- Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous Inference in General Parametric Models. *Biometrical J.* 50, 346–363. doi:10.1002/bimj.200810425
- Hudson, L.N., Emerson, R., Jenkins, G.B., Layer, K., Ledger,

References

M.E., Pichler, D.E., Thompson, M.S.A., O’Gorman, E.J., Woodward, G., Reuman, D.C., 2013. Cheddar: analysis and visualisation of ecological communities in R. *Methods Ecol. Evol.* 4, 99–104. doi:10.1111/2041-210X.12005

Huerta, B., Jakimska, A., Llorca, M., Ruhí, A., Margoutidis, G., Acuña, V., Sabater, S., Rodriguez-Mozaz, S., Barcelò, D., 2015. Development of an extraction and purification method for the determination of multi-class pharmaceuticals and endocrine disruptors in freshwater invertebrates. *Talanta* 132, 373–381. doi:10.1016/j.talanta.2014.09.017

Huxel, G.R., McCann, K., 1998. Food Web Stability: The Influence of Trophic Flows across Habitats. *Am. Nat.* 152, 460–469. doi:10.1086/286182

Jackson, A.L., Inger, R., Parnell, A.C., Bearhop, S., 2011. Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *J. Anim. Ecol.* 80, 595–602. doi:10.1111/j.1365-2656.2011.01806.x

Jaeger, K.L., Olden, J.D., Pelland, N. a, 2014. Climate change poised to threaten hydrologic connectivity and endemic fishes in dryland streams. *Proc. Natl. Acad. Sci. U. S. A.* 111, 1–6. doi:10.1073/pnas.1320890111

Janauer, G., Dokulil, M., 2006. Macrophytes and algae in running waters, in: Ziglio, G., Siligardi, M., Flaim, G. (Eds.), *Biological Monitoring of Rivers. Applications and Perspectives*. Brussels,

Belgium, pp. 89–110.

Jeffrey, S.W., Humphrey, G.F., 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochem. und Physiol. der Pflanz.* 167, 191–194.

Jennings, S., Blanchard, J.L., 2004. Fish abundance with no fishing: predictions based on macroecological theory. *J. Anim. Ecol.* 73, 632–642.

Katano, I., Negishi, J.N., Minagawa, T., Doi, H., Kawaguchi, Y., Kayaba, Y., 2009. Longitudinal macroinvertebrate organization over contrasting discontinuities: effects of a dam and a tributary. *J. North Am. Benthol. Soc.* 28, 331–351. doi:10.1899/08-010.1

Koniak, G., Noy-Meir, I., 2009. A hierarchical, multi-scale, management-responsive model of Mediterranean vegetation dynamics. *Ecol. Modell.* 220, 1148–1158.

Kraus, J.M., Pomeranz, J.F., Todd, A.S., Walters, D.M., Schmidt, T.S., Wanty, R.B., 2016. Aquatic pollution increases use of terrestrial prey subsidies by stream fish. *J. Appl. Ecol.* 56, 44–53. doi:10.1111/1365-2664.12543

Kuzmanovic, M., Ginebreda, A., Petrovic, M., Barceló, D., 2015. Risk assessment based prioritization of 200 organic micropollutants in 4 Iberian rivers. *Sci. Total Environ.* 503–504, 289–299. doi:10.1016/j.scitotenv.2014.06.056

References

- Laird, N.M., Ware, J.H., 1982. Random-Effects Models for Longitudinal Data. *Biometrics* 38, 963. doi:10.2307/2529876
- Lake, P.S., 2003. Ecological effects of perturbation by drought in owing waters. *Freshw. Biol.* 48, 1161–1172. doi:10.1046/j.1365-2427.2003.01086.x
- Laliberté, E., Legendre, P., 2010. A distance-based framework for measuring functional diversity from multiple traits. *Ecology* 91, 299–305.
- Laliberté, E., Legendre, P., Shipley, B., 2014. FD: measuring functional diversity from multiple traits, and other tools for functional ecology. R package version 1.0-12.
- Lamberti, G.A., 1996. The role of periphyton in benthic food webs, in: Stevenson, R., Bothwell, M., Lowe, R. (Eds.), *Algal Ecology*. San Diego, pp. 533–572.
- Layman, C.A., Arrington, D.A., Montaña, C.G., Post, D.M., 2007. Can stable isotope ratios provide for community wide measures of trophic structure. *Ecology* 88, 42–48. doi:10.1038/news050808-1
- Lobera, G., Muñoz, I., López-Tarazón, J.A., Vericat, D., Batalla, R.J., 2017. Effects of flow regulation on river bed dynamics and invertebrate communities in a Mediterranean river. *Hydrobiologia* 784, 283–304. doi:10.1007/s10750-016-2884-6
- López-Doval, J.C., Ginebreda, A., Caquet, T., Dahm, C.N., Petrovic, M., Barceló, D., Muñoz, I., 2013. Pollution in

mediterranean-climate rivers. *Hydrobiologia* 719, 427–450. doi:10.1007/s10750-012-1369-5

Loreau, M., 2010. *The Challenges of Biodiversity Science*. International Ecology Institute, Oldendorf/Luhe, Germany.

Lytle, D.A., Poff, N.L., 2004. Adaptation to natural flow regimes. *Trends Ecol. Evol.* 19, 94–100. doi:10.1016/j.tree.2003.10.002

Maloney, K.O., Munguia, P., Mitchell, R.M., 2011. Anthropogenic disturbance and landscape patterns affect diversity patterns of aquatic benthic macroinvertebrates. *J. North Am. Benthol. Soc.* 30, 284–295. doi:10.1899/09-112.1

Mandarić, L., Mor, J.-R., Sabater, S., Petrović, M., 2018. Impact of urban chemical pollution on water quality in small, rural and effluent-dominated Mediterranean streams and rivers. *Sci. Total Environ.* 613–614, 763–772. doi:10.1016/j.scitotenv.2017.09.128

Margalef, R., 1986. *Ecologia*. Omega.

Marti, E., Aumatell, J., Godé, L., Poch, M., Sabater, F., 2004. Nutrient Retention Efficiency in Streams Receiving Inputs from Wastewater Treatment Plants. *J. Environ. Qual.* 33, 285–293.

Martinez, A., Larrañaga, A., Miguélez, A., Yvon-Durocher, G., Pozo, J., 2016. Land use change affects macroinvertebrate community size spectrum in streams: the case of *Pinus radiata* plantations. *Freshw. Biol.* 61, 69–79. doi:10.1111/fwb.12680

Mate, I., Barrull, J., Salicrú, M., Ruiz-Olmo, J., Gosàlbez, J., 2013.

References

Habitat selection by Southern water vole (*Arvicola sapidus*) in riparian environments of Mediterranean mountain areas: a conservation tool for the species. *Acta Theriol. (Warsz)*. 58, 25–37. doi:10.1007/s13364-012-0090-0

Maulvault, A.L., Santos, L.H.M.L.M., Camacho, C., Anacleto, P., Barbosa, V., Alves, R., Ferreira, P.P., Serra-Compte, A., Barceló, D., Rodriguez-Mozaz, S., Rosa, R., Diniz, M., Marques, A., 2018. Antidepressants in a changing ocean: Venlafaxine uptake and elimination in juvenile fish (*Argyrosomus regius*) exposed to warming and acidification conditions. *Chemosphere* 209, 286–297. doi:10.1016/j.chemosphere.2018.06.004

McCann, K., 2007. Protecting biostructure. *Nature* 446, 29. doi:10.1038/446029a

McConnaughey, T., McRoy, C.P., 1979. Food-Web Structure and the Fractionation of Carbon Isotopes in the Bering Sea. *Mar. Biol.* 53, 257–262.

Mcgoff, E., Solimini, A.G., Pusch, M.T., Jurca, T., Sandin, L., 2013. Does lake habitat alteration and land-use pressure homogenize European littoral macroinvertebrate communities? *J. Appl. Ecol.* 50, 1010–1018. doi:10.1111/1365-2664.12106

McHugh, P.A., McIntosh, A.R., Jellyman, P.G., 2010. Dual influences of ecosystem size and disturbance on food chain length in streams. *Ecol. Lett.* 13, 881–890. doi:10.1111/j.1461-0248.2010.01484.x

- McIntyre, P.B., Jones, L.E., Flecker, A.S., Vanni, M.J., 2007. Fish extinctions alter nutrient recycling in tropical freshwaters. *Proc. Natl. Acad. Sci.* 104, 4461–4466. doi:10.1073/pnas.0608148104
- Medici, C., Butturini, A., Bernal, S., Vázquez, E., Sabater, F., Vélez, J., Francés, F., 2008. Modelling the non-linear hydrological behaviour of a small Mediterranean forested catchment. *Hydrol. Process.* 22, 3814–3828.
- Merckx, T., Souffreau, C., Kaiser, A., Baardsen, L.F., Backeljau, T., Bonte, D., Brans, K.I., Cours, M., Dahirel, M., Debortoli, N., De Wolf, K., Engelen, J.M.T., Fontaneto, D., Gianuca, A.T., Govaert, L., Hendrickx, F., Higuti, J., Lens, L., Martens, K., Matheve, H., Matthysen, E., Piano, E., Sablon, R., Schön, I., Van Doninck, K., De Meester, L., Van Dyck, H., 2018. Body-size shifts in aquatic and terrestrial urban communities. *Nature* 558, 113–116. doi:10.1038/s41586-018-0140-0
- Meyer, E., 1989. The relationship between body length parameters and dry mass in running water invertebrates. *Arch. für Hydrobiol.* 117, 191–203.
- Miller, R.J., 1966. *Simultaneous Statistical Inference*. McGrawHill Book Co., New York.
- Mittermeier, R.A., Myers, N., Robles Gil, P., Mittermeier, C.G., 1999. *Hotspots: Earth's Biologically Richest and Most Threatened Ecosystems*. Cemex, Mexico, D.F.
- Mondy, C.P., Muñoz, I., Dolédec, S., 2016. Life-history strategies

References

constrain invertebrate community tolerance to multiple stressors: A case study in the Ebro basin. *Sci. Total Environ.* 572, 196–206. doi:10.1016/J.SCITOTENV.2016.07.227

Mor, J.R., Lopez-Doval, J., Muñoz, I., Sabater, S. The fast food syndrome on freshwater ecosystems: urban wastewater affects the fitness of river invertebrates. In Prep.

Murdock, J., Roelke, D., Gelwick, F., 2004. Interactions between flow, periphyton, and nutrients in a heavily impacted urban stream: Implications for stream restoration effectiveness. *Ecol. Eng.* 22, 197–207. doi:10.1016/j.ecoleng.2004.05.005

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27, 31–36. doi:10.1016/S0003-2670(00)88444-5

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. *Nature* 403, 853–858.

Nakano, S., Miyasaka, H., Kuhara, N., 1999a. Terrestrial–Aquatic linkages: Riparian arthropod inputs alter trophic cascades in a stream food web. *Ecology* 80, 2435–2441.

Nilsson, C., Reidy, C.A., Dynesius, M., Revenga, C., 2005. Fragmentation and flow regulation of the world's large river systems. *Science* (80-.). 308, 405–408.

Nilsson, C., Svedmark, M., 2002. *Basic Principles and Ecological*

Consequences of Changing Water Regimes: Riparian Plant Communities. *Environ. Manage.* 30, 468–480. doi:10.1007/s00267-002-2735-2

O'Connor, N., Donohue, I., 2012. Environmental context determines multi-trophic effects of consumer species loss. *Glob. Chang. Biol.* 1–10. doi:10.1111/gcb.12061

Olden, J.D., Comte, L., Giam, X., 2018. The Homogocene: a research prospectus for the study of biotic homogenisation. *NeoBiota* 37, 23–36. doi:10.3897/neobiota.37.22552

Olden, J.D., Comte, L., Giam, X., 2016. Biotic Homogenisation. *eLS* 1–8. doi:10.1002/9780470015902.a0020471.pub2

Ortiz, J.D., Martí, E., Puig, M.À., 2005. Recovery of the macroinvertebrate community below a wastewater treatment plant input in a Mediterranean stream. *Hydrobiologia* 545, 289–302. doi:10.1007/s10750-005-3646-z

Ortiz, J.D., Puig, M. a., 2007. Point source effects on density, biomass and diversity of benthic macroinvertebrates in a Mediterranean stream. *River Res. Appl.* 23, 155–170. doi:10.1002/rra.971

Passy, S.I., Blanchet, F.G., 2007. Algal communities in human-impacted stream ecosystems suffer beta-diversity decline. *Divers. Distrib.* 13, 670–679. doi:10.1111/j.1472-4642.2007.00361.x

Petchey, O.L., Belgrano, A., 2010. Body-size distributions and size-

References

spectra: universal indicators of ecological status? *Biol. Lett.* 6, 434–437. doi:10.1098/rsbl.2010.0240

Petrovic, M., Ginebreda, A., Acuña, V., Batalla, R.J., Elosegi, A., Guasch, H., de Alda, M.L., Marcé, R., Muñoz, I., Navarro-Ortega, A., Navarro, E., Vericat, D., Sabater, S., Barceló, D., 2011. Combined scenarios of chemical and ecological quality under water scarcity in Mediterranean rivers. *TrAC - Trends Anal. Chem.* 30, 1269–1278. doi:10.1016/j.trac.2011.04.012

Pierce, C.L., 1988. Predator avoidance, microhabitat shift, and risk-sensitive foraging in larval dragonflies. *Oecologia* 77, 81–90. doi:10.1007/BF00380929

Pimm, S.L., Lawton, J.H., 1977. Number of trophic levels in ecological communities. *Nature* 268, 329–331.

Piqué, G., Batalla, R.J., Sabater, S., 2015. Hydrological characterization of dammed rivers in the NW Mediterranean region. *Hydrol. Process.* 30, 1691–1707.

Plank, M.J., Law, R., 2011. Ecological drivers of stability and instability in marine ecosystems. *Theor. Ecol.* 5, 465–480. doi:10.1007/s12080-011-0137-x

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. *Freshw. Biol.* 55, 194–205. doi:10.1111/j.1365-2427.2009.02272.x

- Polis, G.A., Hurd, S.D., 1995. Extraordinarily high spider densities on islands: flow of energy from the marine to terrestrial food webs and the absence of predation. *Proc. Natl. Acad. Sci.* 92, 4382–4386.
- Pomeranz, J.P.F., Warburton, H.J., Harding, J.S., 2019. Anthropogenic mining alters macroinvertebrate size spectra in streams. *Freshw. Biol.* 64, 81–92. doi:10.1111/fwb.13196
- Ponsatí, L., Acuña, V., Aristi, I., Arroita, M., García-Berthou, E., von Schiller, D., Elosegi, A., Sabater, S., 2015. Biofilm Responses to Flow Regulation by Dams in Mediterranean Rivers. *River Res. Appl.* 31, 1003–1016.
- Post, D.M., 2002a. The long and short of food-chain length. *Trends Ecol. Evol.* 17, 269–277. doi:10.1016/S0169-5347(02)02455-2
- Post, D.M., 2002b. Using stable isotopes to estimate trophic position: models, methods and assumptions. *Ecology* 83, 703–718.
- Post, D.M., Doyle, M.W., Sabo, J.L., Finlay, J.C., 2007. The problem of boundaries in defining ecosystems: A potential landmine for uniting geomorphology and ecology. *Geomorphology* 89, 111–126.
- Post, D.M., Takimoto, G., 2007. Proximate structural mechanisms for variation in food-chain length. *Oikos* 116, 775–782. doi:10.1111/j.2007.0030-1299.15552.x

References

- Power, M.E., Dietrich, W.E., 2002. Food webs in river networks. *Ecol. Res.* 17, 451–471. doi:10.1046/j.1440-1703.2002.00503.x
- Power, M.E., Dietrich, W.E., Finlay, J.C., 1996. Dams and downstream aquatic biodiversity: Potential food web consequences of hydrologic and geomorphic change. *Environ. Manage.* 20, 887–895. doi:10.1007/BF01205969
- Power, M.E., Holomuzki, J.R., Lowe, R.L., 2013. Food webs in Mediterranean rivers. *Hydrobiologia* 719, 119–136. doi:10.1007/s10750-013-1510-0
- Power, M.E., Parker, M.S., Dietrich, W.E., 2008. Seasonal reassembly of a river food web: Floods, droughts, and impacts of fish. *Ecol. Monogr.* 78, 263–282. doi:10.1890/06-0902.1
- Power, M.E., Sun, A., Parker, G., Dietrich, W.E., Wootton, J.T., 1995. Hydraulic Food-Chain Models. *Bioscience* 45, 159–167. doi:10.2307/1312555
- R Core Team, 2014. R: A language and environment for statistical computing.
- Ramsay, P.M., Rundle, S.D., Attrill, M.J., Uttley, M.G., Williams, P.R., Elsmere, P.S., Abada, A., 1997. A rapid method for estimating biomass size spectra of benthic metazoan communities. *Can. J. Fish. Aquat. Sci.* 54, 1716–1724. doi:10.1139/f97-081
- Reuman, D.C., Mulder, C., Raffaelli, D., Cohen, J.E., 2008. Three allometric relations of population density to body mass:

theoretical integration and empirical tests in 149 food webs. *Ecol. Lett.* 11, 1216–1228. doi:10.1111/j.1461-0248.2008.01236.x

Rice, J., Westerhoff, P., 2017. High levels of endocrine pollutants in US streams during low flow due to insufficient wastewater dilution. *Nat. Geosci.* 10, 587–591. doi:10.1038/NGEO2984

Riede, J.O., Brose, U., Ebenman, B., Jacob, U., Thompson, R., Townsend, C.R., Jonsson, T., 2011. Stepping in Elton's footprints : a general scaling model for body masses and trophic levels across ecosystems. *Ecol. Lett.* 14, 169–178. doi:10.1111/j.1461-0248.2010.01568.x

Rodriguez-Capítulo, A., Muñoz, I., Bonada, N., Gaudes, A., Tomanova, S., 2009. La biota de los ríos: los invertebrados, in: Sabater, S., Elosegui, A. (Eds.), *Conceptos y Técnicas En Ecología Fluvial*. Fundación BBVA, pp. 253–270.

Rohr, J.R., Kerby, J.L., Sih, A., 2006. Community ecology as a framework for predicting contaminant effects. *Trends Ecol. Evol.* 21, 606–613. doi:10.1016/j.tree.2006.07.002

Rooney, N., McCann, K.S., Moore, J.C., 2008. A landscape theory for food web architecture. *Ecol. Lett.* 11, 867–881. doi:10.1111/j.1461-0248.2008.01193.x

Ruhí, A., Acuña, V., Barceló, D., Huerta, B., Mor, J.R., Rodríguez-Mozaz, S., Sabater, S., 2015a. Bioaccumulation and trophic magnification of pharmaceuticals and endocrine disruptors in a Mediterranean river food web. *Sci. Total Environ.* 540, 250–259.

doi:10.1016/j.scitotenv.2015.06.009

Ruhí, A., Holmes, E.E., Rinne, J.N., Sabo, J.L., 2015b. Anomalous droughts, not invasion, decrease persistence of native fishes in a desert river. *Glob. Chang. Biol.* 21, 1482–1496. doi:10.1111/gcb.12780

Ruhí, A., Muñoz, I., Tornés, E., Batalla, R.J., Vericat, D., Ponsatí, L., Acuña, V., von Schiller, D., Marcé, R., Bussi, G., Francés, F., Sabater, S., 2016. Flow regulation increases food-chain length through omnivory mechanisms in a Mediterranean river network. *Freshw. Biol.* 61, 1536–1549. doi:10.1111/fwb.12794

Rundel, P.W., Montenegro, G., Jaksic, F.M., 1998. Land-scape disturbance and biodiversity in Mediterranean-type ecosystems. Springer-Verlag, Berlin (Germany).

Sabater, S., 2008. Alterations of the global water cycle and their effects on river structure, function and services. *Freshw. Rev.* 1, 75–88.

Sabater, S., Bregoli, F., Acuña, V., Barceló, D., Elosegi, A., Ginebreda, A., Marcé, R., Muñoz, I., Sabater-Liesa, L., Ferreira, V., 2018. Effects of human-driven water stress on river ecosystems: a meta-analysis. *Sci. Rep.* 8, 11462. doi:10.1038/s41598-018-29807-7

Sabater, S., Muñoz, I., Feio, M.J., M.Romaní, A., Graça, M.A., 2009. The Iberian Rivers, in: Tockner, K., Uehlinger, U., Robinson, C.T. (Eds.), *Rivers of Europe*. Academic Press, pp. 113–149.

References

- Sabo, J.L., Bastow, J.L., Power, M.E., 2002. Length–mass relationships for adult aquatic and terrestrial invertebrates in a California watershed. *Benthol. Soc* 21, 336–343.
- Sabo, J.L., Finlay, J.C., Kennedy, T., Post, D.M., 2010. The role of discharge variation in scaling of drainage area and food chain length in rivers. *Science* (80-.). 330, 965–967. doi:10.1126/science.1196005
- Sabo, J.L., Post, D.M., 2008. Quantifying periodic, stochastic, and catastrophic environmental variation. *Ecol. Monogr.* 78, 19–40. doi:10.1890/06-1340.1
- Sánchez-Morales, M., Sabater, F., Muñoz, I., 2018. Effects of urban wastewater on hyporheic habitat and invertebrates in Mediterranean streams. *Sci. Total Environ.* 642, 937–945. doi:10.1016/j.scitotenv.2018.06.132
- Schmid-Araya, J.M., Schmid, P.E., Robertson, A., Winterbottom, J., Gjerlov, C., Hildrew, A.G., 2002. Connectance in stream food webs. *J. Anim. Ecol.* 71, 1056–1062. doi:10.1046/j.1365-2656.2002.00668.x
- Shannon, C.E., 1949. The mathematical theory of communication, in: Shannon, C.E., Weaver, W. (Eds.), *The Mathematical Theory of Communication*. University of Illinois Press, Urbana, USA, pp. 29–125.
- Shea, K., Chesson, P., 2002. Community ecology theory as a framework for biological invasions. *Trends Ecol. Evol.* 17, 170–

176. doi:10.1016/s0169-5347(02)02495-3

Shieh, S.H., Kondratieff, B.C., Ward, J. V., 1999. Longitudinal changes in benthic organic matter and macroinvertebrates in a polluted Colorado plains stream. *Hydrobiologia* 411, 191–209. doi:10.1023/A:1003835929331

Socolar, J.B., Gilroy, J.J., Kunin, W.E., Edwards, D.P., 2016. How Should Beta-Diversity Inform Biodiversity Conservation? *Trends Ecol. Evol.* 31, 67–80. doi:10.1016/j.tree.2015.11.005

Sokolova, I.M., Frederich, M., Bagwe, R., Lannig, G., Sukhotin, A.A., 2012. Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. *Mar. Environ. Res.* 79, 1–15.

Spink, A.J., Murphy, K.J., Smith, S.M., Westlake, D.F., 1993. Effects of Eutrophication on *Ranunculus* and *Potamogeton*. *J. Aquat. Plant Manag.* 31, 113–117.

Statzner, B., Bêche, L.A., 2010. Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? *Freshw. Biol.* 55, 80–119. doi:10.1111/j.1365-2427.2009.02369.x

Statzner, B., Bis, B., Dolédec, S., Usseglio-Polatera, P., 2001. Perspectives for biomonitoring at large spatial scales: a unified measure for the functional composition of invertebrate communities in European running waters. *Basic Appl. Ecol.* 2, 73–85.

Statzner, B., Bonada, N., Dolédec, S., 2007. Conservation of taxonomic and biological trait diversity of European stream macroinvertebrates communities: a case for collective public database. *Biodivers. Conserv.* 16, 3609–3632. doi:10.1007/s10531-007-9150-1

Stelzer, R.S., Lamberti, G.A., 2002. Ecological stoichiometry in running waters: Periphyton chemical composition and snail growth. *Ecology* 83, 1039–1051. doi:10.1890/0012-9658(2002)083[1039:ESIRWP]2.0.CO;2

Tachet, H., Richoux, P., Bournaud, M., Usseglio-Polatera, P., 2010. *Invertébrés d'Eau Douce*, 2nd correc. ed. CNRS éditions, Paris.

Tamburello, N., Côté, I.M., Dulvy, N.K., 2015. Energy and the Scaling of Animal Space Use. *Am. Nat.* 186. doi:10.1086/682070

Tank, J.J.L., Rosi-Marshall, E.J.E., Griffiths, N. a., Entekin, S. a., Stephen, M.L., 2010. A review of allochthonous organic matter dynamics and metabolism in streams. *J. North Am. Benthol. Soc.* 29, 118–146. doi:10.1899/08-170.1

Tierno de Figueroa, J.M., López-Rodríguez, M.J., Fenoglio, S., Sánchez-Castillo, P., Fochetti, R., 2013. Freshwater biodiversity in the rivers of the Mediterranean Basin. *Hydrobiologia* 719, 137–186.

Timoner, X., Acuña, V., von Schiller, D., Sabater, S., 2012. Functional responses of stream biofilms to flow cessation desiccation and rewetting. *Freshw. Biol.* 57, 1565–1578.

References

- Tornés, E., Mor, J.R., Mandaric, L., Sabater, S., 2018. Diatom responses to sewage inputs and hydrological alteration in Mediterranean streams. *Environ. Pollut.* 238, 369–378. doi:10.1016/j.envpol.2018.03.037
- Tornés, E., Sabater, S., 2010. Variable discharge alters habitat suitability for benthic algae and cyanobacteria in a forested Mediterranean stream. *Mar. Freshw. Res.* 61, 441. doi:10.1071/MF09095
- Trenberth, K.E., 2011. Changes in precipitation with climate change. *Clim. Res.* 47, 123–138.
- Uehlinger, U., 2000. Resistance and resilience of ecosystem metabolism in a flood-prone river system. *Freshw. Biol.* 45, 319–332. doi:10.1111/j.1365-2427.2000.00620.x
- Vander Zanden, M.J., Shuter, B.J., Lester, N., Rasmussen, J.B., 1999. Patterns of Food Chain Length in Lakes: A Stable Isotope Study. *Am. Nat.* 154, 406–416. doi:10.1086/303250
- Vannote, R.L., Minshall, W.G., Cummins, K.W., Sedell, J.R., Cushing, C.E., 1980. The River Continuum Concept. *Can. J. Fish. Aquat. Sci.* 37, 130–137. doi:10.1139/f80-017
- Vidal-Abarca, M., Montes, C., Suárez, M., Ramírez-Díaz, L., 1990. Sectorización ecológica de cuencas fluviales: aplicación a la cuenca del río Segura (SE España). *An. Geogr. la Univ. Complut.* 10, 149–182.

References

- Voelz, N.J., Ward, J. V., 1991. Biotic Responses along the Recovery Gradient of a Regulated Stream. *Can. J. Fish. Aquat. Sci.* 48, 2477–2490. doi:Doi 10.1139/F91-289
- von Schiller, D., Aristi, I., Ponsatí, L., Arroita, M., Acuña, V., Elozegi, A., Sabater, S., 2016. Regulation causes nitrogen cycling discontinuities in Mediterranean rivers. *Sci. Total Environ.* 540, 168–177. doi:10.1016/j.scitotenv.2015.07.017
- Vörösmarty, C.J., Green, P., Salisbury, J., Lammers, R.B., 2000. Global Water Resources: Vulnerability from Climate Change and Population Growth. *Science* (80-.). 289, 284–288.
- Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, a, Green, P., Glidden, S., Bunn, S.E., Sullivan, C. a, Liermann, C.R., Davies, P.M., 2010. Global threats to human water security and river biodiversity. *Nature* 467, 555–561. doi:10.1038/nature09549
- Wetzel, R.G., 1983. *Periphyton of freshwater ecosystems*. Dr. W. Junk Publishers, The Hague.
- White, E.P., Ernest, S.K.M., Kerkhoff, A.J., Enquist, B.J., 2007. Relationships between body size and abundance in ecology. *Trends Ecol. Evol.* 22. doi:10.1016/j.tree.2007.03.007
- Williams, R.J., 2010. *Network3D Software*. Microsoft Research, Cambridge, UK.
- Winemiller, K.O., McIntyre, P.B., Castello, L., Fluet-Chouinard,

References

E., Giarrizzo, T., Nam, S., Baird, I.G., Darwall, W., Lujan, N.K., Harrison, I., Stiassny, M.L.J., Silvano, R.A.M., Fitzgerald, D.B., Pelicice, F.M., Agostinho, A.A., Gomes, L.C., Albert, J.S., Baran, E., Petrere, M., Zarfl, C., Mulligan, M., Sullivan, J.P., Arantes, C.C., Sousa, L.M., Koning, A.A., Hoeinghaus, D.J., Sabaj, M., Lundberg, J.G., Armbruster, J., Thieme, M.L., Petry, P., Zuanon, J., Vilara, G.T., Snoeks, J., Ou, C., Rainboth, W., Pavanelli, C.S., Akama, A., van Soesbergen, A., Saenz, L., 2016. Balancing hydropower and biodiversity in the Amazon, Congo, and Mekong. *Science* (80-.). 351, 128–129. doi:10.1126/science.aac7082

Wolman, M.G., 1954. A method of sampling coarse river-bed material. *Trans. Am. Geophys. Union* 35, 951. doi:10.1029/TR035i006p00951

Woodward, G., Hildrew, A.G., 2002a. Food web structure in riverine landscapes. *Freshw. Biol.* 47, 777–798.

Woodward, G., Hildrew, A.G., 2002b. Body-size determinants of niche overlap and intraguild predation within a complex food web. *J. Anim. Ecol.* 71, 1063–1074. doi:10.1046/j.1365-2656.2002.00669.x

Woodward, G., Warren, P., 2006. Body size and predatory interactions in freshwaters: scaling from individuals to communities, in: Hildrew, A. G., Raffaelli, D., Edmonds-Brown, R. (Eds.), *Body Size: The Structure and Function of Aquatic Ecosystems*. pp. 98–117.

Wootton, J.T., 1994. The Nature and Consequences of Indirect Effects in Ecological Communities. *Annu. Rev. Ecol. Syst.* 25, 443–466.

Yandell, B.S., 1997. Practical data analysis for designed experiments. Chapman & Hall.

Yoon, I., Williams, R., Levine, E., Yoon, S., Dunne, J., Martinez, N., 2004. Webs on the Web (WOW): 3D visualization of ecological networks on the WWW for collaborative research and education, in: Erbacher, R.F., Chen, P.C., Roberts, J.C., Gröhn, M.T., Bärner, K. (Eds.), *Proceedings of the IS&T/SPIE Symposium on Electronic Imaging*. pp. 124–132. doi:10.1117/12.526956

Yvon-durocher, G., Allen, A.P., 2012. Linking community size structure and ecosystem functioning using metabolic theory. *Philos. Trans. R. Soc.* 367, 2998–3007. doi:10.1098/rstb.2012.0246

Yvon-Durocher, G., Montoya, J.M., Trimmer, M., Woodward, G., 2011. Warming alters the size spectrum and shifts the distribution of biomass in freshwater ecosystems. *Glob. Chang. Biol.* 17, 1681–1694. doi:10.1111/j.1365-2486.2010.02321.x

Zarfl, C., Lumsdon, A.E., Berlekamp, J., Tydecks, L., Tockner, K., 2016. A global boom in hydropower dam construction. *Aquat. Sci.* 77, 161–170. doi:10.1007/s00027-014-0377-0

Supplementary Material

This thesis generated a big database, to avoid over expanding the printed document this information was reduced, but could be found on the joint CD or obtained on the supplementary material of each published paper or contacting with the authors.

Supplementary Materials for

Chapter 1: Dam regulation and riverine food-web structure in a Mediterranean river

This document includes:

Appendix S1.1 Non-animal content volume-mass ratios.

Table S1.1 Vertebrate and invertebrate diet composition.

Table S1.2 Abundance of diatom species.

Table S1.3 Abundance of non-diatom algae.

Table S1.4 Abundance of vertebrate and invertebrate taxa.

***Complete information about food-web links between pairs of taxa could be found in Table S5 in the published paper online version.**

Supporting Information: Appendix S1

Non-animal content mass-volume ratios. The volume of non-animal gut content was estimated using a compound microscope (80 x). The gut structure was carefully removed, and its contents were dried at 60°C during 24h, and weighted in a micrometric precision balance ($\pm 10^{-6}$ mg). Samples were analyzed individually and grouped taxonomically: Diptera (Orthoclaadiinae and Simuliidae), Gasteropoda (*Ancylus fluviatilis*), and Plecoptera and Ephemeroptera (*Nemoura* sp. and *Caenis* sp.). Median values were selected to transform volume to weight.

Taxa	N	Median (mg·mm ⁻³)
Diptera	49	1.57381
Gasteropoda	30	0.06704
Plecoptera and Ephemeroptera	62	0.34104

		Diet content (%)													
		Non-animal material (< 0.02 mm)													
Consumer	Site	N	Inv	Fish	Ter	TOTAL	Dead Animal	Bacteria	Detritus	Diatoms	Fil. algae	Fungi	Non- fil. algae	Vegetal material	Category
	D2	3	99.7	0	0	0.3	NA	NA	NA	NA	NA	NA	NA	NA	Insectovor
	D3	1	100	0	0	0	0	0	0	0	0	0	0	0	Insectovor
<i>Ancylus fluviatilis</i>	U1	20	0	0	0	100	2.78	0.00	49.62	20.71	2.97	2.01	11.60	10.32	Detritivor
	D1	2	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Herbivor ⁴
	D3	12	0	0	0	100	7.58	0.00	3.71	73.21	2.93	0.52	3.71	8.35	Herbivor
<i>Antocha</i> sp	D1	6	0	0	0	100	36.59	0	1.22	36.59	12.20	12.20	0	1.22	Herbivor
	D2	4	0.4	0	0	99.6	0	0	2.26	67.90	22.63	2.26	2.26	2.26	Herbivor
<i>Boyeria irene</i>	D2	1	100	0	0	0	0	0	0	0	0	0	0	0	Insectovor
Brachyptera	U1	8	0	0	0	100	1.58	0	11.44	47.25	1.58	0	33.37	4.79	Herbivor
	D2	8	0	0	0	100	4.17	0	41.67	41.67	4.17	4.17	0	4.17	Herbivor
	D3	1	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Herbivor ⁴
<i>Caenis</i> sp.	D1	20	0	0	0	100	20.12	0	25.48	20.12	2.73	2.73	0	28.83	Herbivor
	D2	20	0	0	0	100	13.44	0	18.84	18.22	17.84	5.05	0	26.61	Herbivor
	D3	20	0	0	0	100	12.40	0	26.29	19.84	13.25	12.40	0.50	15.30	Herbivor
<i>Centroptilum luteolum</i>	U1	1	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Herbivor ⁴
	D1	1	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Herbivor ⁴
Chimarra	D2	1	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Detritivor ⁴
	D3	1	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Detritivor ⁴
<i>Chironomus</i> sp.	D3	1	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Detritivor ⁴
<i>Ephemerella</i> sp	D3	20	9.8	0	0	90.2	1.33	0.37	41.03	35.83	2.16	3.93	1.19	4.33	Herbivor
<i>Habrophlebia</i> sp	D3	4	0	0	0	100	0	0	25	25	25	0	0	25	Herbivor
<i>Hydropsyche</i> sp	D1	1	56.5	0	0	43.5	NA	NA	NA	NA	NA	NA	NA	NA	Insectovor
	D2	2	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Herbivor ⁴
Isoperla	U1	14	94.3	0	0	0	0.23	0	2.35	2.35	0.23	0.00	0.23	0.23	Insectovor
	D3	1	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Herbivor ⁴
<i>Leuctra cf inermis</i>	D1	1	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Herbivor ⁴
Limnephilus	U1	4	12.6	0	0	87.4	2.29	0	20.64	20.64	0.23	11.47	20.64	11.47	Herbivor

Diet content (%)															
Non-animal material (< 0.02 mm)															
Consumer	Site	N	Inv	Fish	Ter	TOTAL	Dead Animal	Bacteria	Detritus	Diatoms	Fil. algae	Fungi	Non- fil. algae	Vegetal material	Category
<i>Limnius</i> sp	U1	1	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Herbivor ⁴
	D1	1	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Herbivor ⁴
	D2	4	0	0	0	100	0	0	32.26	32.26	0	32.26	3.23	0	Herbivor
Lumbriculidae	U1	1	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Detritivor ⁴
	D2	4	0	0	0	100	42.86	0	23.81	14.29	0	14.29	0	4.76	Detritivor
	D3	4	0	0	0	100	22.52	0	31.53	31.53	0.45	13.51	0	0.45	Detritivor
Naididae	D2	8	0	0	0	100	85.36	0	6.17	1.15	1.15	0	0	6.17	Detritivor
	D3	20	0	0	0	100	61.31	0	1.64	22.76	3.16	0.23	1.81	9.09	Detritivor
Nemoura	U1	20	46.2	0	0	53.8	12.19	0	20.48	10.16	0.56	0.63	6.68	3.09	Insectovor
<i>Onychogomphus cf uncatius</i>	D1	4	100	0	0	0	0	0	0	0	0	0	0	0	Insectovor
	D2	4	100	0	0	0	0	0	0	0	0	0	0	0	Insectovor
	D3	2	100	0	0	0	0	0	0	0	0	0	0	0	Insectovor
Orthocladinae	U1	20	0	0	0	100	31.88	0	31.88	26.88	0	8.80	0	0.56	Detritivor
	D1	20	0	0	0	100	14.959	0	15.71	26.46	0	9.94	0	32.93	Herbivor
	D2	20	0	0	0	100	0	0	30.88	14.65	12.40	0	11.19	30.88	Herbivor
	D3	20	0	0	0	100	15.12	0	12.88	36.64	14.90	6.09	1.49	12.88	Herbivor
Ostracoda	U1	15	0	0	0	3.5	30.162	0	30.16	23.45	6.71	0	0	6.04	Detritivor
<i>Oulimnius rivularis</i> (Adult)	U1	7	0	0	0	100	33.33	0	33.33	0	0	0	0	33.33	Detritivor
<i>Oulimnius rivularis</i> (Larva)	D2	6	0	0	0	100	0	0	0	0	0	0	0	100	Herbivor
<i>Oxygastra curtisii</i>	D3	1	100	0	0	0	0	0	0	0	0	0	0	0	Insectovor
<i>Palpomyia</i> sp.	U1	1	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Detritivor ⁴
	D1	2	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Detritivor ⁴
<i>Perlodes</i> sp.	U1	6	100	0	0	0	0	0	0	0	0	0	0	0	Insectovor
<i>Platycnemis</i> sp.	D2	3	100	0	0	0	0	0	0	0	0	0	0	0	Insectovor
Plectrocnemia	D1	1	100	0	0	0	0	0	0	0	0	0	0	0	Insectovor
<i>Polycentropus</i> sp.	D2	3	100	0	0	0	0	0	0	0	0	0	0	0	Insectovor
Prosimulium	U1	16	2.0	0	0	98.0	15.32	0.00	21.40	54.59	1.23	1.82	1.82	1.82	Herbivor

Diet content (%)

Non-animal material (< 0.02 mm)

Consumer	Site	N	Inv	Fish	Ter	TOTAL	Non-animal material (< 0.02 mm)								Category
							Dead Animal	Bacteria	Detritus	Diatoms	Fil. algae	Fungi	Non-fil. algae	Vegetal material	
<i>Radix</i> sp.	D1	1	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Herbivor ⁴
<i>Rhabdiopteryx</i> sp	U1	3	0	0	0	100	2.27	0	2.27	68.18	2.27273	0	2.27	22.73	Herbivor
<i>Simullium</i>	U1	16	0	0	0	100	22.74	0	21.14	28.69	0	20.92	3.26	3.26	Herbivor
	D3	20	0.1	0	0	99.9	1.61	0	22.23	57.49	11.02	2.99	2.99	1.61	Herbivor
<i>Stenelmis</i> sp. (Adult)	D3	1	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Herbivor ⁴
<i>Stenelmis consobrina</i>	D2	2	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Herbivor ⁴
Tanipodinae	U1	1	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Detritivor ⁴
Tanitarsinii	U1	1	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Detritivor ⁴
	D1	20	0	0	0	100	59.91	0	9.12	13.27	2.07	0	0	15.63	Detritivor
Tanypodinae	D2	2	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Detritivor ⁴
Tinodes	D3	2	0	0	0	100	NA	NA	NA	NA	NA	NA	NA	NA	Herbivor ⁴
Tipulidae	D3	2	3.1	0	0	96.9	NA	NA	NA	NA	NA	NA	NA	NA	Herbivor ⁴
Tubificidae	U1	20	0	0	0	100	88.95	0	8.89	1.23	0.47	0	0	0.47	Detritivor

References

- de Sostoa, A., Lobon-Cervia, J., 1989. Observations on feeding relationships between fish predators and fish assemblages in a Mediterranean stream. *Regul. Rivers Res. Manag.* 4, 157–163. doi:10.1002/rrr.3450040208
- Rugiero, L., Capula, M., [...] Angelici, F.M., 2000. Life-history and diet of two populations of *Natrix maura* (Reptilia, Colubridae) from contrasted habitats in Sardinia. *Miscel·lània Zoològica* 23, 41–51.
- Ruhí, A., Muñoz, I., [...], Sabater, S., 2016. Flow regulation increases food-chain length through omnivory mechanisms in a Mediterranean river network. *Freshw. Biol.* 61, 1536–1549. doi:10.1111/fwb.12794
- Tachet, H., Richoux, P., Bournaud, M., Usseglio-Polatera, P., 2002. *Invertébrés d'Eau Douce*, 2nd correc. ed. CNRS éditions, Paris.

Table S1.2 Abundance (% of occurrence) of diatom species at each study site.

Taxon	Site U1	Site D1	Site D2	Site D3
<i>Achnanthes biasolettiana</i> Grun. var. thienemannii (Hustedt) Lange-Bertalot	0.00	0.00	1.07	0.00
<i>Achnanthes biasolettiana</i> Grunow	0.00	30.41	14.00	0.00
<i>Achnanthes biasolettiana</i> Grunow var. subatomus Lange-Bertalot	0.00	0.00	3.44	0.00
<i>Achnanthes clevei</i> Grunow	0.00	0.00	0.12	0.00
<i>Achnanthes lanceolata</i> (Breb.) Grunow	0.97	0.00	0.00	0.00
<i>Achnanthes lanceolata</i> (Breb.) Grun. ssp. frequentissima Lange-Bertalot	0.72	0.00	0.00	0.24
<i>Achnanthes minutissima</i> Kützing	24.40	33.33	43.65	66.59
<i>Achnanthes rupestoides</i> Hohn	0.00	0.00	0.00	0.96
<i>Achnanthes straubiana</i> Lange-Bertalot	0.00	0.12	0.00	0.00
<i>Achnanthes subhudsonis</i> Hustedt	0.00	0.12	0.00	0.00
<i>Achnanthes</i> sp.	0.00	0.00	0.36	0.00
<i>Amphora inariensis</i> Krammer	0.00	0.00	0.47	0.72
<i>Amphora pediculus</i> (Kützing) Grunow	0.00	3.04	2.85	3.61
<i>Caloneis bacillum</i> (Grunow) Cleve	0.00	0.12	0.24	0.72
<i>Cocconeis pediculus</i> Ehrenberg	0.00	0.23	0.12	0.24
<i>Cocconeis placentula</i> Ehrenberg var. euglypta (Ehr.) Grunow	0.00	0.00	0.36	0.00
<i>Cocconeis placentula</i> Ehrenberg var. lineata (Ehr.) Van Heurck	0.00	0.23	0.24	0.00
<i>Cyclotella comta</i> (Ehr.) Kützing	0.00	0.12	0.12	0.00
<i>Cyclotella ocellata</i> Pantocsek	0.00	0.82	0.95	0.00
<i>Cymbella excisa</i> Kützing	0.00	0.58	0.71	2.88
<i>Cymbella excisiformis</i> Krammer var. excisiformis	0.00	0.00	0.71	0.00
<i>Cymbella caespitosa</i> (Kützing) Brun	0.00	0.00	0.36	0.48
<i>Cymbella hustedtii</i> Krasske	0.00	0.00	0.00	0.24
<i>Cymbella lacustris</i> (Agardh) Cleve	0.00	0.00	0.12	0.00
<i>Cymbella lange-bertalotii</i> Krammer	0.00	0.00	0.12	0.00
<i>Cymbella microcephala</i> Grunow	0.00	15.91	16.84	4.57
<i>Cymbella minuta</i> Hilse ex Rabenhorst	0.00	0.82	0.83	0.72
<i>Cymbella neoleptoceros</i> Krammer	0.00	0.12	0.12	0.00
<i>Cymbella prostrata</i> (Berkeley) Grunow	0.00	0.00	0.00	0.24
<i>Cymbella silesiaca</i> Bleisch	0.00	0.12	0.24	0.00
<i>Cymbella vulgata</i> Krammer	0.00	0.00	0.12	0.00
<i>Denticula kuetzingii</i> Grunow	0.00	0.12	0.00	0.00
<i>Denticula tenuis</i> Kützing	0.00	3.51	0.47	0.00
<i>Diatoma moniliformis</i> Kützing	0.24	0.00	0.00	0.00
<i>Diatoma vulgare</i> Bory 1824	0.00	0.23	0.12	0.24
<i>Diploneis oblongella</i> (Naegeli) Cleve-Euler	0.00	0.00	0.24	0.00
<i>Epithemia sorex</i> Kützing	0.00	0.00	0.00	0.48
<i>Fragilaria brevistriata</i> Grunow	0.00	0.23	0.12	0.00
<i>Fragilaria capucina</i> Desmazières var. vaucheriae (Kützing) Lange-Bertalot	0.00	0.00	0.36	0.00
<i>Fragilaria capucina</i> Desmazières var. gracilis (Oestrup) Hustedt	0.00	0.12	0.00	0.24
<i>Fragilaria capucina</i> Desmazières var. mesolepta (Rabenhorst) Rabenhorst	0.00	0.35	0.12	0.00
<i>Fragilaria capucina</i> Desmazières var. radians (Kützing) Lange-Bertalot	0.00	0.23	0.00	0.00
<i>Fragilaria capucina</i> var. perminuta (Grunow) Lange-Bertalot	0.00	0.94	0.00	0.00
<i>Fragilaria capucina</i> Desmazières var. rumpens (Kützing) Lange-Bertalot	0.00	1.87	2.14	0.24
<i>Fragilaria construens</i> f. binodis (Ehr.) Hustedt	0.00	0.35	0.12	0.24
<i>Fragilaria pinnata</i> Ehrenberg	0.00	0.58	0.59	0.72
<i>Fragilaria ulna</i> (Nitzsch.) Lange-Bertalot	0.00	0.12	0.00	0.00

Supplementary Material: Chapter 1

Taxon	Site U1	Site D1	Site D2	Site D3
<i>Gomphonema clavatum</i> Ehr.	0.00	0.00	0.00	0.24
<i>Gomphonema micropus</i> Kützing	0.97	0.00	0.00	0.00
<i>Gomphonema minutum</i> (Ag.) Agardh	0.00	0.00	0.00	0.24
<i>Gomphonema olivaceum</i> (Hornemann) Brébisson	64.49	0.23	0.00	0.72
<i>Gomphonema parvulum</i> Kützing	0.00	0.00	0.12	0.00
<i>Gomphonema truncatum</i> Ehr.	0.00	0.23	0.12	0.24
<i>Gyrosigma attenuatum</i> (Kützing) Rabenhorst	0.00	0.12	0.00	0.00
<i>Navicula atomus</i> (Kütz.) Grunow var. <i>permitis</i> (Hustedt) Lange-Bertalot	0.24	0.00	0.00	0.00
<i>Navicula capitatoradiata</i> Germain	0.00	0.12	0.24	0.48
<i>Navicula cryptotenella</i> Lange-Bertalot	0.00	0.47	0.36	0.24
<i>Navicula cryptotenelloides</i> Lange-Bertalot	0.00	1.17	3.91	1.92
<i>Navicula gregaria</i> Donkin	0.24	0.00	0.00	0.00
<i>Navicula menisculus</i> Schuman var. <i>grunowii</i> Lange-Bertalot	0.00	0.00	0.00	0.24
<i>Navicula minima</i> Grunow	0.00	0.00	0.00	0.48
<i>Navicula radiosa</i> Kützing	0.00	0.00	0.12	0.24
<i>Navicula reichardtiana</i> Lange-Bertalot	0.24	0.23	0.12	1.92
<i>Navicula saprophila</i> Lange-Bertalot & Bonik	0.24	0.00	0.00	0.00
<i>Navicula stroemii</i> (Hustedt) Mann	0.00	0.00	0.47	0.00
<i>Navicula subalpina</i> Reichardt	0.00	0.00	0.24	0.00
<i>Navicula subhamulata</i> (Grunow in V. Heurck) D.G. Mann	0.00	0.00	0.12	0.00
<i>Navicula tripunctata</i> (O.F.Müller) Bory	0.00	0.12	0.00	0.00
<i>Navicula veneta</i> Kützing	0.00	0.00	0.12	0.00
<i>Nitzschia acidoclinata</i> Lange-Bertalot	0.00	0.00	0.12	0.00
<i>Nitzschia acula</i> Hantzsch	0.00	0.23	0.00	0.00
<i>Nitzschia amphibia</i> Grunow	0.00	0.12	0.00	0.24
<i>Nitzschia aurariae</i> Chohnoky	0.00	0.12	0.00	0.00
<i>Nitzschia dissipata</i> (Kützing) Grunow	0.00	0.35	0.24	0.24
<i>Nitzschia fonticola</i> Grunow	2.42	0.47	0.47	6.73
<i>Nitzschia lacuum</i> Lange-Bertalot	0.00	0.23	0.47	1.20
<i>Nitzschia linearis</i> (Agardh) W.M.Smith	0.00	0.12	0.12	0.00
<i>Nitzschia microcephala</i> Grunow in Cleve & Moller	0.00	0.00	0.00	0.24
<i>Nitzschia palea</i> (Kützing) W. Smith	0.00	0.35	0.00	0.00
<i>Nitzschia paleacea</i> (Grunow) Grunow in van Heurck	4.83	0.00	0.00	0.00
<i>Nitzschia recta</i> Hantzsch in Rabenhorst	0.00	0.12	0.12	0.00
<i>Nitzschia supralitorea</i> Lange-Bertalot	0.00	0.23	0.00	0.00
<i>Nitzschia aff. vermicularis</i> (Kützing)Hantzsch	0.00	0.00	0.12	0.00
<i>Rhoicosphenia abbreviata</i> (C.Agardh) Lange-Bertalot	0.00	0.12	0.00	0.00
<i>Simonsenia delognei</i> Lange-Bertalot	0.00	0.12	0.00	0.00

Table S1.3 Abundance (% of occurrence) of non-diatom algae found at each sampling site, compared to total Bacillariophyta (diatom) occurrence.

Group	Taxon	Site U1	Site D1	Site D2	Site D3
Bacillariophyta	Bacillariophyta	35.71	20.41	14.81	8.33
Cyanobacteria	<i>Xenococcus kernerii</i>	21.43	6.12	0.00	0.00
	<i>Pleurocapsa minor</i>	7.14	6.12	0.00	0.00
	<i>Phormidium faveolarum</i>	7.14	0.00	0.00	0.00
	<i>Homoeothrix janthina</i>	7.14	0.00	0.00	0.00
	<i>Homoeothrix</i> sp.1	0.00	4.08	0.00	0.00
	<i>Homoeothrix</i> sp.2	0.00	2.04	0.00	0.00
	<i>Homoeothrix crustacea/varians</i>	0.00	4.08	0.00	0.00
	<i>Gloeocapsa</i> sp.1	0.00	2.04	0.00	0.00
	<i>Gloeocapsa</i> sp.2	0.00	0.00	3.70	0.00
	<i>Phormidium</i> sp.	0.00	0.00	5.56	4.17
	<i>Pseudoanabaena</i> sp.	0.00	2.04	0.00	0.00
	chroococcal (aff. <i>Microcystis</i>)	0.00	6.12	7.41	8.33
	<i>Chroococcus</i> sp.	0.00	2.04	5.56	2.08
	<i>Nostoc verrucosum</i>	0.00	10.20	0.00	0.00
	<i>Schizothrix affinis</i>	0.00	8.16	12.96	10.42
	<i>Schizothrix</i> sp.	0.00	8.16	5.56	4.17
	<i>Lyngbya</i> sp.	0.00	0.00	5.56	0.00
	<i>Lyngbya maior</i>	0.00	0.00	5.56	0.00
	<i>Calothrix</i> sp.	0.00	0.00	5.56	0.00
	<i>Calothrix parietina</i>	0.00	0.00	0.00	8.33
	<i>Aphanocapsa rivularis</i>	0.00	0.00	0.00	6.25
Chlorophyta	<i>Gongrosira debaryana</i>	21.43	6.12	3.70	4.17
	<i>Scenedesmus ellipticus</i>	0.00	6.12	3.70	0.00
	<i>Scenedesmus</i> sp.	0.00	0.00	1.85	0.00
	<i>Cladophora glomerata</i>	0.00	2.04	0.00	0.00
	<i>Oedogonium</i> sp.	0.00	0.00	0.00	6.25
	<i>Microspora tumidula</i>	0.00	2.04	0.00	6.25
	<i>Microspora pachyderma</i>	0.00	0.00	0.00	4.17
Streptophyta	<i>Spirogyra</i> sp.1	0.00	2.04	9.26	10.42
	<i>Spirogyra</i> sp.2	0.00	0.00	0.00	10.42
	<i>Mougeotia</i> sp.	0.00	0.00	5.56	6.25
Rhodophyta	<i>Chantransia</i>	0.00	0.00	3.70	0.00

Table S1.4 Abundance (ind./m²) of vertebrate and invertebrate taxa found in each study site.

Group	Taxon	Site U1	Site D1	Site D2	Site D3
Anura	<i>Pelophylax perezii</i>	0.02	0.00	0.00	0.02
Anguilliformes	<i>Anguilla anguilla</i>	0.00	0.01	0.02	0.00
Cypriniformes	<i>Barbus haasi</i>	0.00	0.05	0.08	0.21
	<i>Parachondrostoma miegii</i>	0.00	1.24	1.32	1.70
	<i>Gobio lozanoi</i>	0.00	0.55	0.21	0.05
	<i>Luciobarbus graellsii</i>	0.00	0.19	0.43	0.26
Salmonidae	<i>Salmo trutta</i>	0.00	0.01	0.00	0.00
Squamata	<i>Natrix maura</i>	0.01	0.00	0.00	0.01
Oligochaeta	Lumbriculidae	2	0	8	8
	Naididae	0	0	16	3320
	Tubificidae	308	0	0	0
Gastropoda	<i>Ancylus fluviatilis</i>	130	4	0	28
	<i>Bythinia</i> sp.	0	6	0	0
	<i>Radix</i> sp.	0	2	0	0
Ephemeroptera	<i>Caenis luctuosa</i>	0	116	248	220
	<i>Centroptilum luteolum</i>	2	2	10	210
	<i>Ephemerella</i> sp.	0	0	0	254
	<i>Habrophlebia</i> sp.	0	2	0	8
Plecoptera	<i>Brachyptera</i> sp.	16	0	0	0
	<i>Isoperla</i> sp.	28	0	0	2
	<i>Leuctra cf inermis</i>	0	14	0	0
	<i>Nemoura</i> sp.	278	0	0	0
	<i>Perlodes</i> sp.	12	0	0	0
	<i>Rhabdiopteryx</i> sp.	4	0	0	0
Odonata	<i>Aeshna cf mixta</i>	0	2	6	2
	<i>Boyeria irene</i>	0	0	2	0
	<i>Onychogomphus cf uncatus</i>	0	8	8	4
	<i>Oxygastra curtisii</i>	0	0	0	8
	<i>Platycnemis</i> sp.	0	0	0	6
Heteroptera	<i>Mesovelgia</i> sp.	2	0	0	0
Coleoptera	<i>Agabus</i> sp.	4	0	0	0
	<i>Graptodytes flavipes</i>	4	0	0	0
	<i>Limnius</i> sp.	2	4	8	0
	<i>Oulimnius rivularis</i>	30	8	14	88
	<i>Stenelmis consobrina</i>	0	0	4	2
Trichoptera	<i>Chimarra</i> sp.	0	0	2	2
	<i>Hydropsyche</i> sp.	0	4	4	0
	<i>Limnephilus</i> sp.	8	2	0	0
	<i>Philopotamus montanus</i>	0	2	0	0
	<i>Plectrocnemia</i> sp.	0	2	0	0
	<i>Polycentropus</i> sp.	0	0	10	0

Group	Taxon	Site U1	Site D1	Site D2	Site D3
Diptera	<i>Tinodes</i> sp.	0	0	0	4
	<i>Antocha</i> sp.	0	12	8	0
	<i>cf. Arctopelopia</i> sp.	0	2	0	0
	<i>Atherix</i> sp.	0	0	0	2
	<i>Chaetocladius</i>	0	0	164	0
	<i>Chironomus</i> sp.	0	2	2	2
	<i>Cladotanytarsus</i> sp.	0	2	0	0
	<i>Clinotanytus</i> sp.	0	0	4	0
	<i>Corynoneura</i> sp.	56	0	0	218
	<i>Cricotopus</i> sp.	112	1800	246	1524
	Empididae	0	2	0	0
	<i>Eukiefferiella</i> sp.	0	1136	0	0
	<i>Nanocladius</i> sp.	0	0	0	218
	<i>Nilotanytus</i> sp.	0	10	22	0
	<i>cf. Orthocladius-cricotopus</i>	0	0	0	1088
	<i>Orthocladius</i> sp.	734	718	818	872
	<i>Palpomyia</i> sp.	2	8	0	0
	<i>Parametriocnemus</i> sp.	56	454	246	1088
	<i>Paratrichocladius</i> sp.	170	286	0	0
	<i>Prosimulium</i> sp.	32	0	0	0
	<i>Simulium</i> sp.	32	0	0	140
	<i>Synorthocladius</i> sp.	0	0	164	0
	<i>Tanytarsus</i> sp.	2	16	0	0
Tipulidae	0	0	0	4	
<i>Tvetenia</i> sp.	56	180	0	0	
<i>Virgatanytarsus</i> sp.	0	20	0	0	
Copepoda	Cyclopoida	24	8	6	2
Trombidiformes	Hydracarina	0	28	14	8
Ostracoda		34	2	0	0

Supplementary Materials for

Chapter 2: Invertebrate community responses to urban wastewater effluent pollution under different hydro-morphological conditions

This document includes:

Table S2.1 River substrate grain size (%).

Table S2.2 Trait, categories and code used in the study.

Table S2.3 Water level.

Table S2.4 LMM, Tukey's HSD details for analyses in results section.

Table S2.5 Hydrological, nutrient and pharmaceuticals concentration temporal variability.

Table S2.6 Tukey's HSD test cobble-dominated streams.

Table S2.7 Tukey's HSD test sand-dominated streams.

Table S2.8 Traits affected by sampling periods.

Table S2.1 River substrate size (%). Rivers were classified as “cobble” if > 50% of their streambed was covered by grain size > 64 mm and as “sand” if > 50% of the streambed was covered by < 2 mm grain size.

Site	Reach	>512	512-265	265-128	128-64	64-32	32-16	16-8	8-2	< 2	Classified	Mean Discharge	Mean Flow velocity
Bisbal de Falset	Upstream		4	52	23	16	3			2	Cobble	0.036	0.139
	Downstream		2	28	40	20	5			5	Cobble	0.032	0.198
Bot Canaleta	Upstream					5	5	10		80	Sand	0.035	0.076
	Downstream					2	5	8		85	Sand	0.042	0.312
Bot Gandesa	Upstream					2	1	2		95	Sand	0.004	0.107
	Downstream					6	4	15	15	60	Sand	0.008	0.124
Caseres	Upstream	5	4	16	59	5	1			10	Cobble	0.145	0.063
	Downstream	8	3		79					10	Cobble	0.108	0.022
Corbera d'Ebre	Upstream					2	3		5	90	Sand	0.012	0.050
	Downstream				1	2	18	5	14	60	Sand	0.022	0.181
Maella	Upstream	10	5	20	55	8		1		1	Cobble	0.029	0.060
	Downstream	10	2	10	55	14	4	4		1	Cobble	0.031	0.061
Nonasp	Upstream	8	2	10	60	12	3	2		3	Cobble	0.061	0.152
	Downstream			15	50	30	3			2	Cobble	0.066	0.219
Poboleda	Upstream		5	10	50	15	10	5		5	Cobble	0.039	0.036
	Downstream		5	10	40	20	10	10		5	Cobble	0.010	0.019
Prades	Upstream				5	10	15	10	5	55	Sand	0.003	0.012
	Downstream				5	5	10	15	5	60	Sand	0.014	0.054
Prat de Comte	Upstream						5	10	15	70	Sand	0.003	0.036
	Downstream					2	2			96	Sand	0.018	0.071
Reguers	Upstream		10	10	40	15		5	5	10	Cobble	0	0
	Downstream		10	10	40	20	5	5		10	Cobble	0.001	0.034
Vallderoures	Upstream	10		20	40		5	10	5	10	Cobble	0.313	0.167
	Downstream			10	50	20	10	5		5	Cobble	0.343	0.184

Table S2.2 Trait, categories and code used in the study.

Trait	Category	Code
Maximum potential size (mm)	≤2.5	SIZE1
	2.5-5	SIZE2
	5-10	SIZE3
	10-20	SIZE4
	20-40	SIZE5
	40-80	SIZE6
	>80	SIZE7
Life duration (y.)	≤ 1	LDSH
	>1	LDLO
Maximum number of reproductive cycles per year	<1	SEMIVO
	1	UNIVO
	>1	PLURIVO
Aquatic stages	egg	EGG
	larva	LARVA
	nymph	NYMPH
	imago	IMAGO
Reproductive technique	Ovoviviparity	OVOV
	Isolated eggs, free	FREEGG
	Isolated eggs, cemented	CEMEGG
	Clutches, cemented or fixed	CEMCLU
	Clutches, free	FRECLU
	Clutches in vegetation (endophytic)	CLUVEG
	Clutches, terrestrial	CLUTER
	Asexual	ASEXU
Dispersal	Aquatic passive	AQUPAS
	Aquatic active	AQUACT
	Aerial passive	AERPAS
	Aerial active	AERACT
Resistance forms	Eggs, statoblasts, gemmules	RFEGG
	Cocoons	RFCOC
	Cells against desiccation	RFCELL
	Diapause or dormancy	RFDIAP
	None	RFNON
Respiration of aquatic stages	Tegument	TEG
	Gill	GILL
	Plastron	PLAS
	Spiracle (aerial)	SPIR
Locomotion and attachment to substrate	Flier	FLIER
	Surface swimmer	SURSWI
	Swimmer	SWIMM
	Crawler	CRAW
	Burrower (epibenthic)	BURR
	Interstitial (endobenthic)	INTST
	Attached	ATTACH
Food types	Fine sediment + microorganisms	FSMIC
	Detritus < 1 mm	DELE1
	Plant detritus ≥ 1 mm	PDMO1
	Living microphytes	LIMIC
	Living macrophytes	LIMAC
	Dead animal > 1 mm	DAMO1
	Living microinvertebrates	LIMIV
	Living macroinvertebrates	LIMAV
	Vertebrates	VERT
Feeding habits	Deposit feeder	DEFEE
	Shredder	SHR
	Scraper	SCR
	Filter-feeder	FIFEE
	Piercer (plant or animal)	PIER
	Predator (carver/engulfer/swallower)	PRED

Table S2.4 LMM, Tukey's HSD details for analyses in results section (see acronyms in the text)

Variable		Estimate	Std. Error	p-value
H ₆₀		0.2178	1.1943	0.855
LTWD ₆₀		0.0021	0.01685	0.897
N-NH ₄ ⁺		-3.6504	0.7443	< 0.0001
N-NO ₃ ⁻		0.2474	0.3845	0.52
N-NH ₄ ⁺				
	Summer	-0.813404	0.2866395	0.0517
	Autumn	-1.2401988	0.2866395	<0.001
	Spring	-1.1495524	0.2866395	<0.001
"Toxic"				
	Summer	-4.0107	0.56115	< 0.0001
	Autumn	-4.90648	0.56115	< 0.0001
	Spring	-4.34286	0.56115	< 0.0001
"Harmful toxic"				
	Summer	-1.8795	0.5409	< 0.001
	Autumn	-3.277	0.5409	< 0.001
	Spring	-3.7893	0.5409	< 0.001
CdS "very toxic"		-1.41097	0.25986	< 0.0001
SdS "very toxic"				
	Summer	-0.78132	0.50866	0.64088
	Autumn	-2.68454	0.50866	< 0.001
	Spring	-1.94457	0.50866	0.00183
Species Turnover		0.36832	5.993	<0.001
Richness				
	CdS	-6.824	1.804	< 0.001
	SdS	-4.2	1.865	0.0243
EPT richness				
	CdS	-4.176	1.25	< 0.001
	SdS	-2	0.7865	0.011
Shannon diversity				
	CdS	-0.9424	0.1772	< 0.0001
	SdS	-0.5958	0.2827	0.0351
Abundance				
	CdS	0.4452	0.1718	0.00957
	SdS	-0.23374	0.12841	0.0687
Functional diversity				
	CdS	0.5477	0.1333	< 0.0001
	SdS	-0.5373	0.2746	0.0504

Table S2.5 Hydrological index, nutrient and pharmaceutical concentrations on the three sampling periods by studied location. Nutrient concentrations are given in $\text{mg}\cdot\text{L}^{-1}$ and pharmaceuticals in $\text{ng}\cdot\text{L}^{-1}$.

Stream	Site	Substrate	Campaign	Nutrients			Pharmaceutical concentration			Hydrology	
				N-NH ₄	N-NO ₃	PT	Very toxic	Toxic	Harmful Toxic	LTWD ₆₀	H ₆₀
Bisbal de F	Upstream	Cobble	Autumn 2015	0.006	0.212	0.013	0.000	8.609	7.976	-0.021	-2.016
			Summer 2015	0.008	0.347	0.026	0.246	5.319	21.530	-0.016	-2.684
			Spring 2016	0.001	0.112	0.011	0.000	17.310	9.210	0.044	-1.285
	Downstream	Cobble	Autumn 2015	0.031	0.248	0.039	1.346	39.542	30.242	-0.021	-1.882
			Summer 2015	0.028	0.269	0.072	0.246	10.614	45.323	-0.016	-2.417
			Spring 2016	0.009	0.153	0.025	0.246	20.799	44.958	0.044	-1.265
Canaleta	Upstream	Sand	Autumn 2015	0.013	0.213	0.006	0.000	9.356	21.232	-0.038	-1.221
			Summer 2015	0.001	1.236	0.007	0.246	25.448	5.989	0.047	-3.279
			Spring 2016	0.001	1.147	0.002	0.000	1.910	2.309	0.018	-0.933
	Downstream	Sand	Autumn 2015	0.083	0.808	0.070	2.379	1022.873	65.146	-0.051	-1.425
			Summer 2015	0.001	1.147	0.017	0.246	384.807	8.620	0.063	-2.729
			Spring 2016	2.688	1.143	0.148	0.246	646.837	57.776	0.030	-0.824
Gandesa	Upstream	Sand	Autumn 2015	0.009	1.017	0.007	0.000	1.930	8.464	-0.022	-0.018
			Summer 2015	0.006	0.389	0.008	0.000	4.270	5.989	0.009	-1.304
			Spring 2016	0.001	0.514	0.002	0.545	5.860	7.452	0.022	-2.261
	Downstream	Sand	Autumn 2015	3.408	0.947	0.270	14.342	1846.323	2680.162	-0.012	4.757
			Summer 2015	0.931	0.747	0.097	0.430	477.873	161.683	0.008	-0.592
			Spring 2016	0.136	0.532	0.037	15.668	918.934	1252.803	0.009	-2.033
Caseres	Upstream	Cobble	Autumn 2015	0.004	6.135	0.002	0.246	7.446	10.806	-0.068	-2.892
			Summer 2015	0.001	8.026	0.006	0.314	11.708	6.328	0.080	-3.564
			Spring 2016	0.001	1.806	0.009	0.000	1.910	11.279	0.015	-0.868
	Downstream	Cobble	Autumn 2015	0.022	6.269	0.018	0.791	140.900	77.377	-0.045	-0.629
			Summer 2015	0.001	7.548	0.018	0.314	270.996	32.509	0.058	-3.174
			Spring 2016	0.009	1.819	0.004	0.246	227.844	22.549	0.010	-0.881

Stream	Site	Substrate	Campaign	Nutrients			Pharmaceutical concentration			Hydrology	
				N-NH ₄	N-NO ₃	PT	Very toxic	Toxic	Harmful Toxic	LTWD ₆₀	H ₆₀
Corbera	Upstream	Sand	Autumn 2015	0.011	9.392	0.878	6.881	154.591	330.189	-0.005	-0.130
Corbera	Upstream	Sand	Summer 2015	0.013	9.756	0.433	8.729	129.439	299.072	0.028	-1.291
			Spring 2016	0.019	4.868	0.818	3.512	81.487	216.400	-0.006	-0.713
			Autumn 2015	3.403	7.187	1.435	50.398	4096.469	4064.174	-0.011	2.807
	Downstream	Sand	Summer 2015	1.149	8.840	0.628	26.373	3019.306	1087.555	0.023	-0.841
			Spring 2016	2.929	7.573	0.968	23.217	3279.333	2236.546	0.009	-0.940
Maella	Upstream	Cobble	Autumn 2015	0.009	3.538	0.003	0.000	4.492	11.566	-0.015	1.476
			Summer 2015	0.001	7.433	0.006	0.000	8.508	6.455	0.024	-1.646
			Spring 2016	0.001	2.991	0.002	0.000	4.413	12.301	0.001	-1.185
	Downstream	Cobble	Autumn 2015	5.188	1.121	0.554	26.299	228.070	446.403	-0.014	1.504
			Summer 2015	2.637	1.048	0.463	0.744	107.103	21.492	0.027	-1.615
			Spring 2016	0.102	1.507	0.032	1.447	105.807	245.132	-0.006	-0.407
Nonasp	Upstream	Cobble	Autumn 2015	0.010	0.873	0.003	0.068	1.752	9.693	-0.053	-2.213
			Summer 2015	0.003	3.498	0.004	0.000	24.675	13.136	0.124	-2.743
			Spring 2016	0.001	1.073	0.002	0.000	18.044	8.176	0.004	-0.737
	Downstream	Cobble	Autumn 2015	1.694	0.012	0.189	0.954	121.280	2111.245	-0.042	-1.923
			Summer 2015	0.001	3.243	0.036	0.314	158.994	740.534	0.095	-2.654
			Spring 2016	0.047	1.106	0.029	0.246	337.633	1021.050	0.005	-0.718
Poboleda	Upstream	Cobble	Autumn 2015	0.001	0.021	0.017	0.000	2.468	9.245	0.019	1.016
			Spring 2016	0.001	0.018	0.009	0.000	6.050	1.627	0.073	-1.081
	Downstream	Cobble	Autumn 2015	0.231	0.185	0.065	4.611	125.498	183.304	0.010	1.198
			Spring 2016	0.001	0.044	0.030	0.000	57.081	88.526	0.107	-1.122
Prades	Upstream	Sand	Autumn 2015	0.017	2.225	0.043	0.000	2.161	9.616	0.005	-2.792
			Summer 2015	0.017	1.741	0.050	0.000	2.535	16.949	-0.010	0.324
			Spring 2016	0.012	2.945	0.046	0.000	16.797	9.355	0.021	-0.601
	Downstream	Sand	Autumn 2015	3.296	1.041	0.213	24.387	188.872	236.688	-0.008	1.732
			Summer 2015	6.638	1.193	1.096	6.085	75.381	189.218	-0.002	-1.324

Stream	Site	Substrate	Campaign	Nutrients			Pharmaceutical concentration			Hydrology	
				N-NH ₄	N-NO ₃	PT	Very toxic	Toxic	Harmful Toxic	LTWD ₆₀	H ₆₀
Prat de Comte	Upstream	Sand	Spring 2016	2.692	3.198	0.542	4.917	148.824	307.009	0.022	-0.606
			Autumn 2015	0.014	3.262	0.022	0.710	14.094	15.674	-0.016	-0.036
			Summer 2015	0.001	2.636	0.014	0.000	9.221	17.388	0.027	-1.674
Prat de Comte	Downstream	Sand	Spring 2016	0.007	3.850	0.010	0.545	25.541	1.627	-0.003	-0.351
			Autumn 2015	4.821	1.423	0.334	133.524	6904.984	939.269	-0.005	-2.093
			Summer 2015	0.908	1.465	0.125	0.744	7888.526	179.010	-0.066	-0.923
Vallderoures	Upstream	Cobble	Spring 2016	4.362	1.705	0.617	59.463	7153.487	550.082	0.036	3.139
			Autumn 2015	0.006	1.026	0.005	0.246	17.931	29.931	-0.034	2.574
			Summer 2015	0.001	2.622	0.007	0.246	7.009	20.275	-0.001	-2.207
	Downstream	Cobble	Spring 2016	0.001	0.489	0.008	0.000	16.029	2.711	0.035	-0.835
			Autumn 2015	0.559	1.226	0.147	5.257	581.755	604.756	-0.057	1.735
			Summer 2015	0.274	2.963	0.085	0.246	182.822	128.141	-0.044	-1.504
			Spring 2016	0.403	0.744	0.078	1.263	418.129	450.643	0.065	-0.835

Table S2.6 Tukey's HSD test in cobble-dominated streams. Code according Table S2.

Trait	Mean proportion Upstream	Mean proportion Downstream	Estimate	Std. Error	Z value	p-value
Size 1	0.914875725	0.820565656	0.18874	0.03897	4.843	1.28E-06
Size 2	0.993303093	0.98352051	0.0315	0.05179	0.608	0.543
Size 3	0.744976758	0.820050926	-0.12106	0.03508	-3.451	0.000558
Size 4	0.579161705	0.593982289	-0.10261	0.05793	-1.771	0.0765
Size 5	0.316274674	0.388312789	-0.077	0.06734	-1.143	0.253
LDLE1	0.998531341	0.991327429	0.18524	0.06879	2.693	0.00708
LDMO1	0.998553327	0.991405138	-0.18524	0.06879	-2.693	0.00708
SEMIVO	0.397416734	0.228756384	0.17544	0.04744	3.698	0.000217
UNIVO	0.998760419	0.983880136	0.12991	0.03985	3.26	0.00111
PLURIVO	0.999285804	0.990326804	-0.17636	0.03797	-4.645	3.40E-06
EGG	0.910710102	0.854454533	0.12063	0.02998	4.024	5.72E-05
LARVA	0.963825202	0.945783999	0.06214	0.01688	3.683	0.000231
NYMPH	0.811374636	0.864170712	-0.09706	0.03229	-3.006	0.00265
IMAGO	0.66843303	0.746974674	-0.11141	0.03915	-2.846	0.00443
OVOV	0.270488105	0.409129627	-0.15281	0.05135	-2.976	0.00292
FREEGG	0.642533477	0.702923875	-0.08172	0.02648	-3.086	0.00203
CEMEGG	0.94516518	0.904113306	0.1088	0.0552	1.971	0.0487
CEMCLU	0.986615347	0.937821442	0.1904	0.0606	3.142	0.00168
FRECLU	0.271258197	0.477230086	-0.22286	0.05476	-4.07	4.70E-05
CLUVEG	0.283651618	0.132806472	0.15447	0.06676	2.314	0.0207
CLUTER	0.187876796	0.220571146	-0.0334	0.05788	-0.577	0.564
ASEXU	0.323759201	0.521322521	-0.21872	0.05869	-3.727	0.000194
AQUPAS	0.913294202	0.934232114	-0.05477	0.03835	-1.428	0.153
AQUACT	0.867373464	0.885839409	-0.03842	0.02021	-1.9	0.0574
AERPAS	0.834871887	0.869011009	-0.06522	0.02547	-2.561	0.0104
AERACT	0.867373464	0.885839409	-0.03842	0.02021	-1.9	0.0574
RFEGG	0.599118749	0.4272057	0.20341	0.06601	3.082	0.00206
RFcoc	0.657578441	0.858942017	-0.31558	0.06581	-4.796	1.62E-06
RFCELL	0.685215838	0.745843346	-0.08692	0.03034	-2.865	0.00417
RFDIAP	0.706825181	0.70732018	-0.0007171	0.0440649	-0.016	0.987
RFNON	0.999428959	0.964091256	0.23535	0.05729	4.108	3.99E-05
TEG	0.953908094	0.991845157	-0.1766	0.05318	-3.321	0.000898
GILL	0.97209451	0.913701046	0.18175	0.03743	4.856	1.20E-06
PLAS	0.789258178	0.779207253	0.01612	0.02958	0.545	0.586
SPIR	0.512419547	0.520298114	-0.009266	0.033894	-0.273	0.785
FLIER	0.27000671	0.146668991	0.12621	0.02079	6.071	1.27E-09
SURSWI	0.525497709	0.612563707	-0.10602	0.01995	-5.313	1.08E-07
SWIMM	0.84810587	0.877632261	-0.04121	0.03192	-1.291	0.197
CRAW	0.99222304	0.955632614	0.1747	0.04414	3.958	7.56E-05
BURR	0.502337502	0.580139543	-0.09262	0.03843	-2.41	0.0159
INTST	0.511560556	0.586313059	-0.09524	0.04448	-2.141	0.0322
ATTACH	0.536428937	0.521322521	0.01783	0.04104	0.434	0.664
FSMIC	0.23673117	0.284993838	-0.04999	0.01991	-2.511	0.012

Trait	Mean proportion Upstream	Mean proportion Downstream	Estimate	Std. Error	Z value	p-value
DELE1	0.821935017	0.849691532	-0.05058	0.028	-1.806	0.0709
PDMO1	0.696709344	0.706825181	-0.01418	0.01987	-0.714	0.475
LIMIC	0.927735804	0.90708218	0.05202	0.02454	2.12	0.034
MIMAC	0.573850625	0.532202521	0.05004	0.03286	1.523	0.128
DAMO1	0.291026878	0.280101654	0.01146	0.01636	0.7	0.484
LIMIV	0.538705631	0.467004378	0.08304	0.02711	3.063	0.00219
LIMAV	0.643605607	0.690803688	-0.06346	0.05159	-1.23	0.219
DEFEE	0.834926928	0.914147519	-0.16539	0.03671	-4.505	6.64E-06
SHR	0.905093687	0.881957807	0.05159	0.03121	1.653	0.0983
SCR	0.882898699	0.840442892	0.08401	0.04134	2.032	0.0421
FIFEE	0.427476927	0.36580723	0.06715	0.04978	1.349	0.177
PIER	0.423313898	0.407486508	0.01731	0.05995	0.289	0.773
PRED	0.612721779	0.615878064	-0.004021	0.051203	-0.079	0.937

Table S2.7 Tukey's HSD test in sand-dominated streams. Code according Table S2.

Trait	Mean proportion Upstream	Mean proportion Downstream	Estimate	Std. Error	Z value	p-value
Size 1	0.774728694	0.660824934	0.16437	0.07975	2.061	3.93E-02
Size 2	0.95798793	0.944451699	0.04398	0.10862	0.405	0.686
Size 3	0.791268418	0.825041371	-0.05739	0.08705	-0.659	0.51
Size 4	0.579981271	0.664176636	-0.10769	0.06403	-1.682	0.0926
Size 5	0.635224373	0.676662796	-0.05492	0.15228	-0.361	0.718
LDLE1	0.936411856	0.968627359	-0.1074	0.1944	-0.552	0.581
LDMO1	0.936411875	0.968627358	0.1074	0.1944	0.552	0.581
SEMIVO	0.201277071	0.067266638	0.13534	0.05488	2.466	0.0137
UNIVO	0.985822548	0.956665862	0.12688	0.09474	1.339	0.181
PLURIVO	0.992372808	0.959249074	-0.16288	0.09905	-1.644	1.00E-01
EGG	0.870325411	0.776351898	0.16701	0.07315	2.283	2.24E-02
LARVA	0.934386534	0.956514662	-0.06828	0.04792	-1.425	0.154
NYMPH	0.809413509	0.888188838	-0.1502	0.1083	-1.387	0.165
IMAGO	0.797223023	0.731164106	0.10265	0.07495	1.37	0.171
OVOV	0.416728362	0.430460155	-0.01516	0.07606	-0.199	0.842
FREEGG	0.68565563	0.639642947	0.06147	0.07156	0.859	0.39
CEMEGG	0.882657556	0.774854113	0.195	0.1061	1.838	0.066
CEMCLU	0.955922058	0.943173856	0.04073	0.09279	0.439	0.661
FRECLU	0.372166867	0.604186549	-0.2674	0.09269	-2.885	3.92E-03
CLUVEG	0.154614352	0.072684628	0.08249	0.07172	1.15	0.25
CLUTER	0.176829253	0.262228728	-0.08757	0.08576	-1.021	0.307
ASEXU	0.491468197	0.550937286	-0.06971	0.11169	-0.624	0.533
AQUPAS	0.978289373	0.991418706	-0.07766	0.13661	-0.568	0.57
AQUACT	0.847607034	0.790438441	0.09993	0.05158	1.937	0.0527
AERPAS	0.825488926	0.847266342	-0.03975	0.10024	-0.397	0.692
AERACT	0.642834183	0.607120014	0.04576	0.10992	0.416	0.677
RFEGG	0.285166561	0.121737951	0.1671	0.0835	2.002	0.0453
RFcoc	0.862314309	0.878174056	-0.03221	0.12238	-0.263	7.92E-01
RFCELL	0.720524842	0.669218764	0.0714	0.09741	0.733	0.464
RFDIAP	0.666874777	0.663844169	0.00406	0.10669	0.038	0.97
RFNON	0.973188825	0.986361059	-0.06674	0.09375	-0.712	4.77E-01
TEG	0.999998607	0.998601562	-0.05456	0.14001	-0.39	0.697
GILL	0.867519887	0.780579089	0.1546	0.066	2.342	1.92E-02
PLAS	0.704545079	0.648264111	0.07648	0.12137	0.63	0.529
SPIR	0.512806441	0.620768037	-0.1313	0.133	-0.987	0.324
FLIER	0.106425765	0.044024447	0.06259	0.04334	1.444	1.49E-01
SURSWI	0.574985479	0.611962344	-0.04595	0.0778	-0.591	5.55E-01
SWIMM	0.86967019	0.829120832	0.077	0.08201	0.939	0.348
CRAW	0.861624387	0.81642719	0.08327	0.15401	0.541	5.89E-01
BURR	0.651543878	0.677831146	-0.03519	0.06112	-0.576	0.565
INTST	0.720908128	0.786627825	-0.1002	0.1309	-0.766	0.444
ATTACH	0.470150419	0.497189874	-0.0309	0.05371	-0.575	0.565
FSMIC	0.382771216	0.384210191	-0.001558	0.069479	-0.022	0.982

Trait	Mean proportion Upstream	Mean proportion Downstream	Estimate	Std. Error	Z value	p-value
DELE1	0.88542987	0.937452476	-0.12782	0.08817	-1.45	0.147
PDMO1	0.710755123	0.657439211	0.07316	0.08688	0.842	0.4
LIMIC	0.890808941	0.878007813	0.02744	0.03349	0.819	0.413
MIMAC	0.488367232	0.466383321	0.02502	0.07049	0.355	0.723
DAMO1	0.249594133	0.240474329	0.009407	0.069432	0.135	0.892
LIMIV	0.433052185	0.424262873	0.009728	0.069798	0.139	0.889
LIMAV	0.556610695	0.475036456	0.0953	0.09076	1.05	0.294
DEFEE	0.9681094	0.995342002	-0.1567	0.1406	-1.114	2.65E-01
SHR	0.885139446	0.854329271	0.06253	0.09934	0.63	0.529
SCR	0.795289638	0.702547752	0.06253	0.09934	0.63	0.529
FIFEE	0.342440762	0.401228342	-0.06334	0.07473	-0.848	0.397
PIER	0.233425869	0.102733097	0.1327	0.0851	1.559	0.119
PRED	0.412005264	0.501335123	-0.10049	0.08364	-1.201	0.23

Table S2.8 Traits affected by sampling periods. List of traits which relative use increased or decreased downstream wastewater effluents in dry periods (summer and autumn 2015) compared with the wet period (spring 2016).

Site	Increase	Decrease
Bisbal	Endophytic clutches	
Canaleta	Asexual reproduction Endophytic clutches Food: Fine sediment Aquatic stages: imago Locomotion: endobenthic Piercer Life duration (>1 y.)	Aerial active Aerial passive Crawler Filter-feeder Reproduction: Clutches, free Life duration (≤ 1 y.) Living macrophytes Living macroinvertebrates Living microinvertebrates Aquatic stages: nymph Reproductive: Ovoviviparity Food: Plant detritus ≥ 1 mm Respiration: Plastron Predator Resistance forms: Cells Resistance forms: Diapause Shredder Respiration: Spiracle Locomotion: Surface swimmer
Gandesa	Crawler Food: Dead animal > 1 mm FRECLU Life duration (≤ 1 y.) Life duration (>1 y.) Living macrophytes Living macroinvertebrates Living microinvertebrates Resistance forms: Diapause Scraper Maximum potential size: 5-10 mm	Asexual reproduction Reproduction: Cemented eggs Food: Fine sediment Food: Plant detritus ≥ 1 mm Respiration: Plastron Resistance forms: Cells size1 Respiration: Spiracle Locomotion: Surface swimmer
Corbera	Food: Dead animal > 1 mm Reproduction: Clutches, free Resistance forms: Diapause Maximum potential size: 10-20 mm	Asexual reproduction Food: Fine sediment
Maella	Endophytic clutches	
Nonasp	Locomotion: endobenthic	
Prades	Asexual reproduction Reproduction: Clutches, free Locomotion: endobenthic	Respiration: Spiracle
Prat de Comte	Locomotion: endobenthic	Respiration: Plastron