EFFECTS OF GLOBAL CHANGE ON FISH ASSEMBLAGES IN MEDITERRANEAN STREAMS

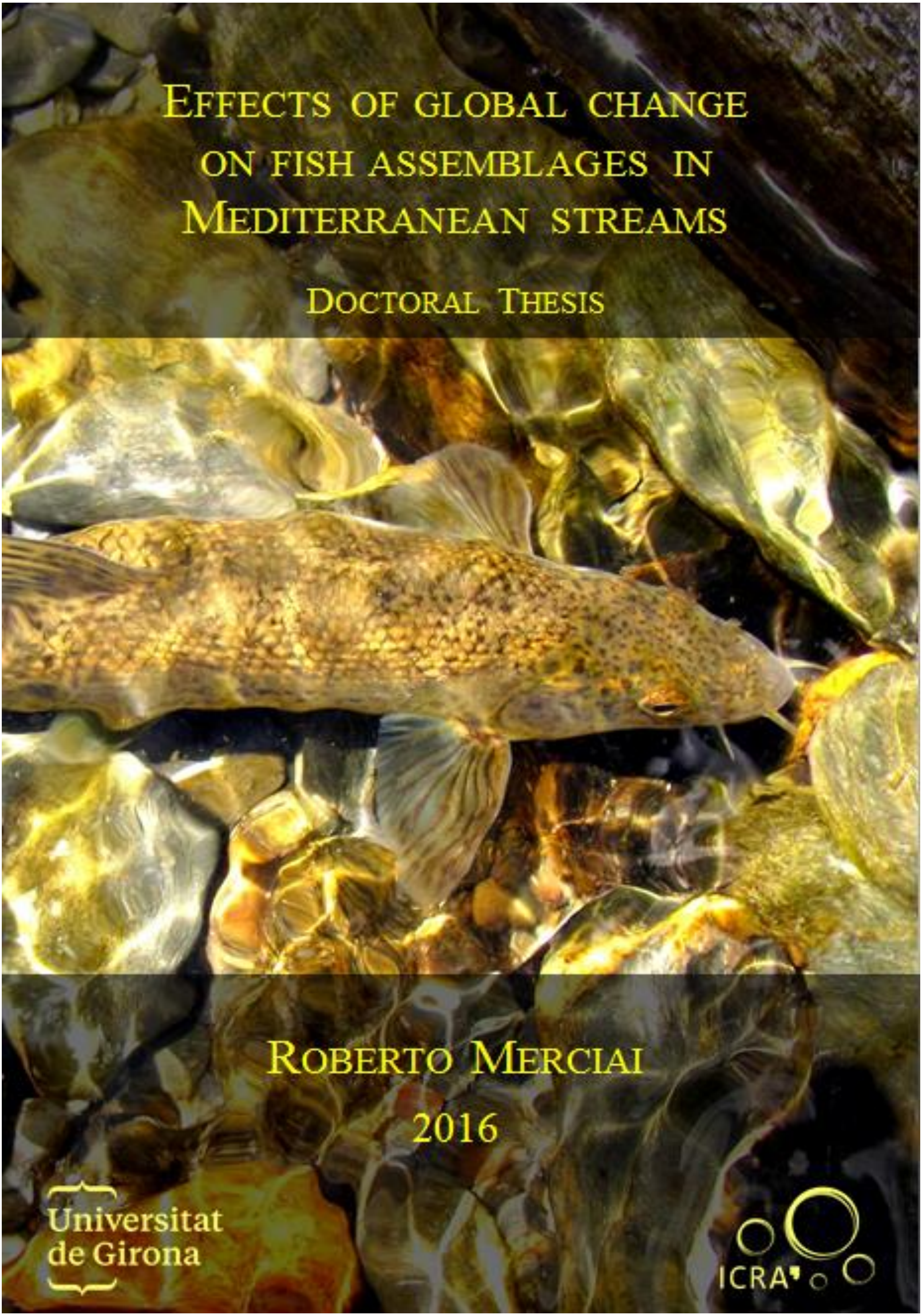
Roberto Merciai

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/10803/405192

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.



EFFECTS OF GLOBAL CHANGE ON FISH ASSEMBLAGES IN MEDITERRANEAN STREAMS

DOCTORAL THESIS

ROBERTO MERCIAI

2016

Universitat de Girona

ICRA

EFFECTS OF GLOBAL CHANGE ON FISH ASSEMBLAGES IN MEDITERRANEAN STREAMS

DOCTORAL THESIS

ROBERTO MERCIAI

2016

TUTOR:

DR. EMILI GARCÍA-BERTHOU

SUPERVISED BY

DR. EMILI GARCÍA-BERTHOU

DR. SERGI SABATER CORTÉS

DOCTORAL PROGRAM IN WATER SCIENCE AND TECHNOLOGY

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE
DOCTORAL DEGREE AT THE UNIVERSITY OF GIRONA

Dr Emili García-Berthou, of University of Girona

Dr Sergi Sabater Cortés, of University of Girona and Catalan Institute for Water Research (ICRA)

We declare

That the thesis entitled “Effects of global change on fish assemblages in Mediterranean streams”, presented by Roberto Merciai 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.

Signature

Dr. Emili García-Berthou

Dr. Sergi Sabater Cortés

Girona, July 2016

Ai miei genitori

ACKNOWLEDGEMENTS

The people I would like to acknowledge one by one are many and wouldn't fit in these few lines. **Thank you everybody for sharing with me some of the best years of my life.**

I want to say thanks to:

my supervisors, Dr. Emili García-Berthou and Dr. Sergi Sabater Cortés. It is not common to have a great scientist to direct your thesis, and I had two at the same time. **Thank you for believing in me and for teaching me two complementary ways of making science;**

Dr. Kurt D. Fausch, Dr. Larissa L. Bailey and Dr. Kevin R. Bestgen, of Colorado State University, for their fundamental help with MCR methodology and the great time we spent together. **I hope we'll have the chance to meet again;**

"Seminary people" and all UdG colleagues and professors. In particular, in the order we met: Anna, Jordi C., Irene, Laura D, Miquel, Núria A, Mercé, Montse, Núria P, Sergi, Jordi B., Alba, Cesc, David, Giulia, Lorena, Maria, and the many ones who left Girona: Juanita, Lorenzo, Albert, Kit, Pao, Vannak, Amaraa, and so many others; Gigi, Lidia, Martaterry, Rosana and all my ICRA friends, who shared with me my first Catalan period; **amb tots vosaltres he passat moments inoblidables, entre feina, sortides de camp, birres, 'Padules', barracuda parties, sorpreses, vòlei, barraques, correfocs, misteris&llegendes de Girona, escursions entre Gavarres, Pirineu i Costa Brava. Si ara Catalunya és la meva segona casa, us ho dec tot a vosaltres!**

Vorrei poi ringraziare i miei amici, quelli di sempre, in ordine di comparsa nella mia vita: Fabrizio, Leo, Nico, Carlo, Jack, Ambra&Gianni, Marco. Ed ovviamente tutti gli altri con cui per vari motivi ci sentiamo meno, che sono tanti e con cui non ho mai perso i contatti, né ho intenzione di farlo. **Grazie a tutti, per esserci stati sempre e per avermi fatto capire la cosa più importante che ho imparato in questi anni lontano da casa, ovvero che i tuoi amici, quelli veri, restano tali ovunque tu sia, ed ovunque essi siano.**

Un agradecemento especial para Laura, por darme a forza necesaria para seguir o camiño nestes anos nos que compartimos xuntos a fase máis dura, quizáis, para un investigador. **Sen ti non sería o mesmo. Graciñas!**

Un ringraziamento particolare va ai miei genitori, a cui dedico questo lavoro. **La mia formazione scientifica è iniziata molto prima di andare all'università, grazie a voi che mi avete insegnato ad amare la natura e a pensare in modo critico e razionale.**

Per ultimi, ma non certo per importanza, ringrazio i miei nonni, che probabilmente sono quelli che hanno sentito di più la mia mancanza da quando sono partito. **Grazie per supportarmi e per essere sempre stati presenti durante questi anni.**

This thesis has been supported by the Spanish Ministry of Education (FPU doctoral fellowship AP2010-4025), the Spanish Ministry of Economy and Competitiveness (projects CSD2009-00065 and CGL2013-43822-R), the Government of Catalonia (ref. 2014 SGR 484), and the EU FP7 project GLOBAQUA (Grant agreement No. 603629).

LIST OF PUBLICATIONS

Articles derived from this doctoral thesis. Two of them are published in international journals.

- Merciai, R., Guasch, H., Kumar, A., Sabater, S., & García-Berthou, E. (2014). Trace metal concentration and fish size: Variation among fish species in a Mediterranean river. *Ecotoxicology and Environmental Safety*, 107: 154-161.

- Bae M.-J., Merciai R., Benejam L., Sabater S. & García-Berthou E. (2016). Small weirs, big effects: disruption of water temperature regimes with hydrological alteration in a Mediterranean stream. *River Research and Applications,* 32: 309–319.

- Merciai R., Molons-Sierra C., Sabater S., García-Berthou E. Water abstraction affects abundance, size-structure and growth of two threatened cyprinid fishes (*manuscript under revision in the journal Plos One*).

- Merciai R., Bailey L., Bestgen K., Fausch K., Zamora L., Sabater S., García-Berthou E. Water diversion reduces abundance and survival of two Mediterranean cyprinids (*article in preparation*).

LIST OF ABBREVIATIONS

ACA: Catalan Water Agency

AIC: Akaike's Information Criterion

AIC_c: Akaike's Information Criterion adjusted for small sample sizes

Alt: altitude

ANCOVA: analysis of covariance

AT: Air Temperature

AWS: Automatic Weather Station

c: recapture probability

CJS: Cormack-Jolly-Seber

CPUE: Catch Per Unit Effort

CWT: Coded Wire Tag

D: water depth

$\widehat{D}$: estimated population density

df: degrees of freedom

DW: Dry Weight

El: electrofishing

EU: European Union

FL: fish Fork Length

FL_E: effect of fish Fork Length on capture probability by electrofishing

FL_T: effect of fish Fork Length on capture probability by trapping

FR: Flow Regime

GLM: Generalized Linear Model

GPS: Global Positioning System

H_2O_2: hydrogen peroxide

HNO_3: nitric acid

IUCN: International Union for Conservation of Nature

K: number of parameters

KOH: potassium hydroxide

MAM: Minimum Adequate Model

MELM: Mixed-Effects Linear Model

$\widehat{N}$: estimated population abundance

p: capture probability

PIT tags: Passive Integrated Transponder tags

Q: water flow

RD: Robust Design

S: apparent survival probability

SE: Standard Error

SS: Sum of Squares (deviance)

Str: stratum

T: Temperature

t: time

Tr: trapping

UTM: Universal Transverse of Mercator

WF: Water Flow

WT: Water Temperature

WW: Wet Weight

WWTP: Waste Water Treatment Plant

LIST OF FIGURES

METHODOLOGY

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

LIST OF TABLES

METHODOLOGY

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

CONTENTS

SUMMARY

The Mediterranean basin is considered a hot spot of global change and biodiversity at the same time. This densely populated area has a long history of human settlements and its climate characteristics and geographical location, along a gradient between temperate and desert climate regions, generates strong conflicts for water resources between human populations and aquatic ecosystems. Mediterranean river and stream biota has to face natural extreme hydrological events due to precipitation variability. Summer drought, in particular, can be aggravated, in intensity and duration, by high water demand, especially for agriculture.

This thesis aims to explore some aspects of the human impacts on fish populations and other components of stream ecosystems in northeast Iberian Peninsula that suffer direct or indirect consequences of flow reduction due to water diversion. In the first chapter we analyzed the sources of variability, in particular fish size and species, which influence the bioaccumulation of trace metals in fishes of the Llobregat, a polluted, hydrologically-altered Mediterranean river that flows in the highly anthropized province of Barcelona. The following three chapters address the consequences of water diversion on fish populations and water temperature regime of the Tordera, a stream affected by moderate anthropic pressure, inhabited by two threatened cyprinid fishes, the Mediterranean barbel *Barbus meridionalis* and the Catalan chub *Squalius laietanus*. We compared fish metrics at individual (growth rates, somatic condition) and population level (abundance, survival probability) between stream reaches with perennial and artificially intermittent flow regimes, to assess the effects of water scarcity. We also analyzed the effects of water abstraction on water temperature regimes along the Tordera: daily and monthly water temperatures, air-water temperature relationships, and time of the day of minima and maxima.

Our results showed that trace metal bioaccumulation varied significantly among fish species, sizes, chemical elements and sampling sites along the Llobregat, largely masking the gradient in metal pollution. In some cases, flesh metal loads exceeded the UE limits for human consumption. The Tordera temperature regime showed clear upstream-downstream gradients, abruptly disrupted at intermittent reaches and at a waste water treatment plant (WWTP). At the intermittent reaches, daily temperatures were more variable and monthly temperatures were less. At the site affected by the

WWTP, we detected an increased air/water temperature slope, which led to higher summer temperatures than in adjacent sites. Fish abundance and survival probability were drastically lower in the intermittent reaches, especially in the case of chub, whereas growth and somatic condition were influenced in a lesser degree by hydrological alterations. Fish metrics for barbel and chub showed maximum values in the middle stream reaches, downstream the WWTP, where temperatures and nutrient availability were higher.

Overall, our thesis exemplifies the important impacts of global change in Mediterranean freshwater fish and streams and how the responses are complex and species-specific.

RESUM

La conca del Mediterrani es considera un punt calent ("hot spot") de canvi global i biodiversitat al mateix temps. Aquesta zona densament poblada té una llarga història d'assentaments humans. Les seves característiques climàtiques i ubicació geogràfica, al llarg d'un gradient entre regions de clima temperat i desèrtiques, genera forts conflictes pels recursos hídrics entre les poblacions humanes i els ecosistemes aquàtics. La biota fluvial meditarrània ha de enfrontar-se als fenòmens hidrològics extrems naturals a causa de la variabilitat de les precipitacions. La sequera estival, en particular, pot ser agreujada, en intensitat i durada, per l'alta demanda d'aigua, especialment per a l'agricultura.

Aquesta tesi té com a objectiu explorar alguns aspectes dels impactes humans sobre les poblacions de peixos i altres components dels ecosistemes fluvials al nord-est de la Península Ibèrica que pateixen les conseqüències directes o indirectes de reducció de cabal a causa de l'extracció de l'aigua. En el primer capítol es van analitzar les fonts de variabilitat, en particular la mida dels peixos i les espècies, que influeixen en la bioacumulació de metalls traça en peixos del Llobregat, un riu mediterrani que flueix a la zona altament antropitzada de la província de Barcelona. Els següents tres capítols es refereixen a les conseqüències de la extracció de l'aigua sobre les poblacions de peixos i el règim de temperatura de l'aigua de la Tordera, un riu moderadament afectat per pressió antròpica, habitada per dues espècies de ciprínids amenaçats, el barb de muntanya *Barbus meridionalis* i la bagra *Squalius laietanus*. Es van comparar unes mètriques en els peixos a nivell individual (taxes de creixement, condició somàtica) i de població (abundància, la probabilitat de supervivència) entre trams de riu amb règims de cabal perennes i artificialment intermitents, per avaluar els efectes de l'escassetat d'aigua. També es van analitzar els efectes de l'extracció d'aigua en els règims de temperatura de l'aigua al llarg de la Tordera: temperatures diàries i mensuals d'aigua, relacions de temperatura d'aire-aigua, i l'hora de mínima i màxima diaria.

Els nostres resultats van mostrar que la bioacumulació de metalls traça variava significativament entre espècies i mides de peixos, elements químics i punts de mostreig al llarg del Llobregat, amagant en gran mesura el gradient de contaminació per metalls. En alguns casos, les concentracions de metalls en el mùscul superen els límits establerts per la Unió Europea per al consum humà. El règim de temperatura de la Tordera va mostrar clars gradients aigües amunt-aigües avall, bruscament interromputs en els trams

intermitents i aigues avall d’una estació depuradora d'aigües residuals (EDAR). En els trams intermitents, les temperatures diàries eren més variables, i les mensuals ho eren menys. Al lloc afectat per la EDAR, es va detectar un increment del pendent entre temperatura d'aire i aigua, el que va portar a temperatures estivals més altes que als punts adjacents.

L'abundància de peixos i la probabilitat de supervivència eren dràsticament menors en els trams intermitents, especialment en el cas de la bagra, mentre que el creixement i la condició somàtica es van veure menys afectades per l’alteració hidrològica. Les mètriques per al barb i la bagra van mostrar valors màxims en el tram mitjà del riu, aigües avall de la EDAR, on les temperatures i la disponibilitat de nutrients van ser majors.

En resum, aquesta tesi exemplifica els importants impactes del canvi global en els peixos d'aigua dolça i rius mediterranis, i com les respostes poden ser complexes i fortament dependents de l’espècie.

RESUMEN

La cuenca del Mediterráneo se considera un punto caliente de cambio global y biodiversidad al mismo tiempo. Esta zona densamente poblada tiene una larga historia de asentamientos humanos. Sus características climáticas y posición geográfica, a lo largo de un gradiente entre regiones templadas y desérticas, conllevan difíciles conflictos por los recursos hídricos entre las poblaciones humanas y los ecosistemas acuáticos. La biota fluvial mediterránea está sometida a eventos hidrológicos extremos a causa de la variabilidad natural en las precipitaciones. La sequía estival, en particular, puede ser agravada en intensidad y duración por la alta demanda de agua, especialmente para la agricultura.

Esta tesis pretende investigar algunos aspectos de los impactos antrópicos en las poblaciones de peces y otros componentes de los ecosistemas fluviales en la Península Ibérica del noreste, que sufren consecuencias directas o indirectas de la reducción del caudal debido a la extracción de agua. En el primer capítulo se analizaron las fuentes de variabilidad que afectan a la bioacumulación de metales traza, particularmente el tamaño y la especie, en peces del Llobregat, un río mediterráneo que corre por la zona altamente antropizada de la provincia de Barcelona. Los restantes tres capítulos tratan las consecuencias que la extracción de agua tiene sobre las poblaciones de peces y el régimen térmico de la Tordera, un río que se encuentra afectado por una presión antrópica moderada, poblado por dos especies de ciprínidos amenazados, el barbo de montaña *Barbus meridionalis* y el bagre *Squalius laietanus*.

Se compararon métricas de peces a nivel individual (tasas de crecimiento, condición somática) y de población (abundancia, probabilidad de supervivencia) entre tramos de río con regímenes de caudal perennes y artificialmente intermitentes, para evaluar los efectos de la escasez de agua. También se analizaron los efectos de la extracción de agua en el régimen de temperatura a lo largo de la Tordera: temperaturas diarias y mensuales de agua, relaciones entre temperatura de aire y agua, y la hora de mínima y máxima diarias.

Nuestros resultados muestran que la bioacumulación de metales traza varía significativamente entre especies y tamaños de peces, elementos químicos y sitios de muestreo a lo largo del Llobregat, escondiendo en gran medida el gradiente de contaminación por metales. En algunos casos, las concentraciones de metal en el músculo superaban los límites impuestos por la UE para el consumo humano.

El régimen de temperatura de la Tordera mostraba claros gradientes aguas arriba-aguas abajo, bruscamente interrumpidos en los tramos intermitentes y aguas abajo de una estación depuradora de aguas residuales (EDAR). En los tramos intermitentes, las temperaturas diarias eran más variables, y las mensuales lo eran menos. En el sitio afectado por el EDAR, se detectó un incremento de la pendiente entre temperatura de aire y agua, lo que llevó a temperaturas estivales más altas que en los sitios adyacentes.

La abundancia de peces y la probabilidad de supervivencia eran drásticamente menores en los tramos intermitentes, especialmente en el caso del bagre, mientras que el crecimiento y la condición somática se vieron menos afectadas por alteraciones hidrológicas. Las métricas para el barbo y el bagre mostraron valores máximos en los tramos medianos del río, aguas abajo del EDAR, donde las temperaturas y la disponibilidad de nutrientes son mayores.

En general, esta tesis muestra algunos ejemplos de impactos del cambio global en ríos mediterráneos y sus peces, y revela cuán complejas y dependientes de la especie pueden ser las respuestas.

RIASSUNTO

Il bacino mediterraneo è considerato un hot spot, sia di cambiamento globale che di biodiversità. Quest'area densamente popolata ha una lunga storia di insediamenti umani. Le sue caratteristiche climatiche e la sua posizione geografica, lungo un gradiente tra zone temperate e desertiche, fanno sì che si crei una forte competizione per le risorse idriche tra popolazioni umane ed ecosistemi acquatici. Il biota fluviale mediterraneo, infatti, deve far fronte a eventi idrologici estremi, dovuti alla naturale variabilità nelle precipitazioni. La durata e l'intensità della siccità estiva, in particolare, possono essere aggravate dall'alto fabbisogno di acqua, soprattutto per l'agricoltura.

L'obiettivo di questa tesi è di esplorare alcuni aspetti dell'impatto antropico sulle popolazioni ittiche ed altre componenti degli ecosistemi fluviali nella penisola iberica nordorientale, che risentono, direttamente o indirettamente, delle conseguenze della riduzione di portata dovuta al prelievo di acqua. Nel primo capitolo sono stati analizzati i fattori che influiscono sul bioaccumulo di metalli in traccia nei pesci, in particolare la taglia e la specie, nel fiume Llobregat, un corso d'acqua inquinato che attraversa la zona fortemente antropizzata della provincia di Barcellona. I tre capitoli successivi trattano le conseguenze del prelievo di acqua sulle popolazioni di pesci e sul regime termico della Tordera, un corso d'acqua moderatamente interessato dalle attività antropiche, popolato da due specie di ciprinidi di interesse conservazionistico: il barbo mediterraneo *Barbus meridionalis* e il cavedano della Catalogna *Squalius laietanus*. Abbiamo confrontato alcune misure sui pesci a livello individuale (tassi di accrescimento, condizione corporea) e popolazionale (abbondanza, probabilità di sopravvivenza) fra tratti di fiume a regime perenne ed intermittente, per valutare gli effetti della scarsità di acqua. Abbiamo inoltre analizzato gli effetti del prelievo di acqua sulle temperature dell'acqua giornaliere e mensili, sulla relazione tra le temperature atmosferiche e dell'acqua, e sugli orari delle temperature massime e minime.

I nostri risultati mostrano che il bioaccumulo di metalli in traccia varia significativamente tra pesci di diverse specie e dimensioni, tra elementi chimici e punti di campionamento lungo lo Llobregat, camuffando gran parte del gradiente longitudinale di contaminazione. In alcuni casi, le concentrazioni di metalli nel muscolo di pesce superava i limiti imposti dalla UE per il consumo umano. Il regime di temperature della Tordera mostrava gradienti netti da monte a valle, improvvisamente alterati in corrispondenza dei tratti intermittenti e in un punto a valle di un impianto di

trattamento delle acque reflue. Nei tratti intermittenti, le temperature giornaliere erano più variabili, mentre quelle mensili lo erano di meno. Nel punto a valle del depuratore abbiamo riscontrato relazioni più strette tra l'andamento delle temperature dell'acqua e di quelle atmosferiche, che portavano ad un aumento delle temperature estive dell'acqua, in confronto ai siti adiacenti. Le stime di abbondanza e di probabilità di sopravvivenza dei pesci sono nettamente inferiori nei tratti a regime intermittente, specialmente nel caso del cavedano. Al contrario, accrescimento e condizione corporea apparivano influenzati in minor misura dall'alterazione idrologica. Tutte le misure calcolate per barbo e cavedano mostravano valori massimi nel corso medio della Tordera, a valle del depuratore, in presenza di temperature più alte e maggiori quantità di nutrienti.

In conclusione, questa tesi mostra alcuni esempi del forte impatto del cambiamento globale sui corsi d'acqua mediterranei e le relative popolazioni di pesci d'acqua dolce, e di come le conseguenze possano essere complesse e specie-specifiche.

GENERAL INTRODUCTION

Rivers and streams under global environmental change

The hydrological, physical and chemical characteristics of rivers are determined by three co-occurring main factors: geology, climate and land use. Due to human activities, the latter two have suffered modifications: at a local scale, the presence of abundant freshwater has historically attracted human settlements and has also induced radical changes in the nearby lands, expressed progressively by the development of agriculture, industry and urbanization. As a return, subsequent effects have occurred on river hydrology, habitat availability, nutrient cycle, sediment transport, or inputs of toxic compounds (Carpenter et al., 1992; Stevenson and Sabater, 2010). Anthropogenic drivers can directly or indirectly influence the temperature regime of water courses. Temperature is a fundamental physical variable of river ecosystems, which at global scale depends on climate (e.g. air temperature, solar radiation, wind speed, precipitation, evaporation) and at a local scale depends on hydrological features (e.g. discharge, water volume and turbulence, groundwater input) (Caissie, 2006). Consequently, hydrological alteration is in turn expected to produce changes on the temperature regime of rivers.

At a global scale, climate change induced by anthropogenic greenhouse gas emissions is predicted to keep increasing atmospheric average temperatures and extreme meteorological events. As a consequence, water temperatures and the frequency of droughts and floods will increase in many regions, alterations of biogeochemical cycles and sediment transport and loss of biodiversity will occur (Sabater, 2008; Stevenson and Sabater, 2010). These trends are predicted to persist as human population is growing and people is migrating from rural to urban areas, increasing the pressure on the urban ecosystems (Grimm et al., 2008).

The effects of climate and land use changes are expected to be higher in densely populated zones and under ongoing water scarcity. Water is scarce in Mediterranean-climate regions, generally situated along a climatic gradient between temperate and desert climate regions and thus expected to be particularly vulnerable to climate change, especially in terms of precipitation regime (Dallman, 1998). Precipitation irregularly distributed in time and space, heterogeneous topography and high anthropogenic pressures strongly influence hydrology in the Mediterranean regions (Servat, 2003). The hydrological regime of Mediterranean rivers reflects the pattern of precipitation, which is generally scarce in summer, leading to low flows and high water temperatures, and may be very abundant in spring and fall, with consequent high flows and sudden floods (Sabater and Tockner, 2010; Bonada and Resh, 2013).

Extreme hydrological events like drought and floods represent a natural, relatively predictable disturbance that the Mediterranean stream biota has to face every year. For this reason, the organisms inhabiting Mediterranean water courses have developed life-history, physiological and behavioral characteristics to overcome the disturbance (Gasith and Resh, 1999; Lake, 2003). During summer, many small Mediterranean streams experience a severe reduction of flow, with consequent changes in physical and chemical conditions of the aquatic habitat. In case of total drought, the stream populations may become extinct. Persisting isolated pools, on the other hand, may act as refugia for some individuals that will possibly recolonize the stream when the water flow is restored, in case they can survive the harsh conditions that may occur within refugia (Lake, 2003; Magoulick and Kobza, 2003).

Drivers of global change in Mediterranean rivers and streams

The stressors by which global environmental change acts on rivers and streams can be classified in two main groups: i) contaminants, which usually act at local scale and have a direct effect on the physiology of organisms, and ii) habitat alterations, which usually act at broader scale and show indirect effects on organisms (Stevenson and Sabater, 2010). The two main stressors considered in this PhD thesis, trace metals and hydrological alteration, belong respectively to the first and second of these categories.

Trace metal contamination

Contaminants of urban, industrial and agricultural origin are found in water, sediments and organisms of many Mediterranean rivers. Contamination and hydrological alteration are two drivers of global change tightly connected to each other, since the toxic effects of pollutants may be enhanced in case of water scarcity, due to increased concentrations in the aquatic environment (Sabater and Tockner, 2010). Trace metals are among the most dangerous classes of pollutants, as they persist in the ecosystem, accumulating in the organisms across the trophic web, and cannot be degraded (Förstner and Wittmann, 2012). They are naturally present in rocks, soils, water and atmosphere, but they have increased their abundance in the biosphere since the beginning of the industrial era (Candelone et al., 1995; Han et al., 2002). Some metals, like zinc, copper, iron and nickel, have a recognized role in biochemical processes, serving as cofactors and activators of enzyme reactions, or exerting a catalytic property such as prosthetic groups in metalloproteins, and they are commonly named “essential” metals (Mildvan 1970; Clark, 2001). These elements take part in redox reactions, electron transfer and structural functions in nucleic acid metabolism. Conversely, some elements like cadmium, lead and arsenic (“non-essential” metals and metalloids), are strongly poisonous to metal-sensitive enzymes and may disrupt the biochemical processes of organisms. Rivers are especially vulnerable to chemical pollution because of their traditional role as receiving bodies for waste waters (Förstner and Wittmann, 2012). The bioaccumulation of trace metals in aquatic organisms, and especially fish, represents a hazard for the environment and for people feeding on fish.

Hydrological alteration

The strong impact of human activities in the Mediterranean area often extend the duration and increase the intensity of drought (Stevenson and Sabater, 2010): water is needed primarily for irrigation (about 60% of the total) and is abstracted both by diverting surface water from rivers, and pumping ground water (Cudennec et al., 2009; Sabater and Tockner, 2010); climate and land use change have raised the human pressure on water resources in the last decades, and these trends are likely to persist (Sabater and Tockner, 2010). Under this scenario, it is evident how man-induced drought may have much heavier consequences on freshwater biota, exacerbating the effects of disturbance such that the adaptation of organisms may no longer be effective to overcome the stress induced by dryness (Gasith and Resh, 1999). For instance, available refuge habitat may be reduced and more disconnected; harsher physico-chemical conditions, e.g. higher temperatures and nutrient concentration and lower oxygen concentration may occur in refugia; biotic interactions, like predation and competition, may increase (Lake, 2000; Magoulick and Kobza, 2003).

The growing need of water resources, moreover, has led to the impoundment of most Mediterranean rivers, aiming not only to create water supplies to be exploited during the dry season, but also to control floods and produce electricity. The presence of a dam, or a diversion, on a water course, on the one hand may alter the natural flow regime, with consequent changes on the morphological and physico-chemical characteristics of the aquatic habitats (e.g. mesohabitat diversity, sediment and nutrient transport, temperature regime), the dam itself representing an interruption of river continuity. Moreover, the set of ecological alterations occurring downstream of a dam may favour the acclimation of non-native species (Poff et al., 2007; Poff and Zimmermann, 2010).

Hydrological alteration has recognized impacts on the temperature regime of large rivers, especially after dams. Temperature directly affects biogeochemical cycles, ecosystem functioning and the physiology, life history and distribution of aquatic organisms (Allan and Castillo, 2007; Brown et al., 2007). The consequences are well documented (e.g. Dolz et al., 1994; Preece, 2004; Todd et al., 2005; Prats et al., 2010): dams affect temperatures by the release of hypolymnetic waters into the downstream river course. Contrariwise, less information is available about small streams and how their temperatures are affected by water diversion and input from waste water treatment plants (WWTPs), though small water courses represent most of a basin drainage area, and considering that even a modest water input or diversion may drastically alter the stream flow and temperature regime.

Effects of global change on Mediterranean river fish assemblages

Fish are considered to be among the taxonomic groups most negatively affected by habitat loss, and especially hydrological alteration (Poff and Zimmerman, 2010). Occupying high levels in the aquatic trophic webs, fish are strongly affected by changes at lower levels, including the bioaccumulation of contaminants like trace metals. For these reasons, fish are commonly used as indicators of ecological integrity (Carpenter et al., 1992; Grabarkiewicz and Davis, 2008).

Rivers in the Mediterranean region have low freshwater fish species richness, compared with other regions at the same latitude, but a large part of its fauna is endemic and threatened according to IUCN criteria (Smith and Darwall, 2006). The main factors to blame for the worrying conservation status of Mediterranean freshwater fish assemblages are water pollution, hydrological alteration (damming, water diversion and drought) and biological invasions (Darwall, 2009), which in turn may be favored by hydrological alteration (Poff et al., 2007; Poff and Zimmermann, 2010).

Modifications of Mediterranean fish assemblage structure (change of relative abundance and decline of native and sensitive species) due to man-induced flow regime disruption have been documented in numerous studies (e.g. Pires et al., 1999; Aparicio et al., 2000; Magalhães et al., 2007; Benejam et al., 2010, 2014). Conversely, a lesser number of studies have been conducted about the consequences of flow reduction on fish at population and individual level, especially about cyprinids, which represent large part of European fish species.

At present, there is no previous information on the survival probability of Mediterranean freshwater fish or how it is affected by anthropogenic disturbances. The mark-recapture methodology, applied in the last study reported in this dissertation, has been previously used in Mediterranean regions mainly to study fish movement (e.g. Aparicio and de Sostoa, 1998; Pires et al., 2014), but is also an essential tool for estimating natural mortality in fish stocks (Vetter, 1988).

At individual level, habitat quality and availability of trophic resources are responsible of fish health, growth and somatic condition (weight-length relationship), which in turn are known to be affected by physiological stress (Pankhurst and Van Der Kraak, 1997) and disturbances like drought and its associated environmental fluctuations (Spranza and Stanley, 2000; Vila-Gispert et al., 2001; Oliva Paterna et al., 2003a, b; Mas-Martí et al. 2010). Finally, the few data available on the effects on fish growth are mainly about salmonids (e.g. Harvey et al., 2006, 2014; Davidson et al., 2010; Teichert et al., 2010), whereas little is known about Mediterranean cyprinids.

Objectives and hypotheses of this thesis

We conducted four studies on two Mediterranean rivers of Catalonia (NE Spain), the Llobregat and the Tordera, aiming to explore: i) heavy metal pollution, and in particular the bioaccumulation process, in fish at individual level; ii) the effects of hydrological alteration, and especially of water diversion, on the temperature regime of a Mediterranean stream and its fishes, at individual and population level.

More in detail, the present dissertation is structured in four chapters:

Chapter 1. *Trace metal concentration and fish size: variation among fish species in a Mediterranean river* – The objective of this study was to explore how the bioaccumulation of nine metallic elements, plus arsenic, in the muscle tissue of fish species varied with fish size and species in the Llobregat, a river affected by several kinds of anthropic impacts, at four sampling sites located along a slight metal pollution gradient. In addition, we compared the metal concentrations in fish muscle with the limits established by European Union, to evaluate the risk for humans in case of consuming fish from the Llobregat.

We hypothesized that the environmental concentrations of trace metals would affect the concentrations in fish tissues. Since trace metal concentration among sites were comparable, similar muscle loads were expected for each species along the river course, particularly for essential metals, whose excretion should be more efficient. We also expected higher concentrations of heavy metals to occur in bottom-feeding fishes, which often ingest sediments with their food.

Chapter 2. *Small weirs, big effects: disruption of water temperature regime with hydrological alteration in a Mediterranean stream* – We aimed to investigate the consequences of water diversion through a weir, and of water input from a WWTP, on the thermal regime of the Tordera stream, well known from previous research to suffer alterations of its hydrological and thermal regimes due to water diversion and groundwater pumping (Benejam et al., 2010; Bae et al., 2014). Namely, we explored the following aspects: i) alterations of the upstream-downstream gradient in seasonal and daily thermal regimes, and ii) the air-temperature relationship.

Water temperature strongly depends on atmospheric temperature: we hypothesized that an increased or decreased correlation between the two, compared with the control sites, may be a result of hydrological alteration. A decreased air-water correlation may be the result of a strong contribution of groundwater, which has a more constant temperature than surface water, to the residual discharge downstream of the weir. A change of air-water relationship was expected downstream of the WWTP too, since waste water treatment includes extended times of exposure to atmospheric temperatures.

Chapter 3. *Water abstraction affects abundance, size-structure and growth of two threatened cyprinid fishes* -. This study aimed to evaluate the effects of water diversion on two endangered cyprinid species (the Mediterranean barbel *Barbus meridionalis* and the Catalan chub *Squalius laietanus*) by comparing growth and body condition (weight-length relationship) between sites affected by water diversion and control sites.

These metrics are known to be affected by water scarcity and were hypothesized to be lower in the control sites, due to physiological stress and increased competition for food during oversummering, because of harsh physico-chemical conditions and overcrowding in refugia. Moreover, lower sizes are expected in impacted sites, as a consequence of lesser probability of attaining large sizes in circumstances of high seasonal mortality (Magoulick and Kobza, 2003) and size-selective mortality due to physiological stress, i.e. juveniles of some fishes from intermittent streams are known to be better adapted than adults to survive harsh conditions in summer refugia (e.g. Magalhães, 1993; Fernandez-Delgado and Herrera, 1995).

Chapter 4. *Water diversion reduces abundance and survival of two Mediterranean cyprinids.* This chapter is a second part of the study reported in chapter 3 and aims to quantify the effects of water diversion on the same fish species, by comparing survival probability and abundance between impact and control sites.

We expected to find significantly lower survival probabilities in the impact sites, and at all sites in summer, as well as abundance was expected to be much higher at the control sites, which never dry up.

METHODOLOGY

Study area

The study sites of this thesis were situated on two Iberian watercourses, The Llobregat and The Tordera (Figg. 1-2-3). Both are located in Catalonia (NE Spain), flow into the Mediterranean Sea and show typical Mediterranean regimes. As well as most water courses in the same area, these rivers experience high levels of anthropogenic impact.

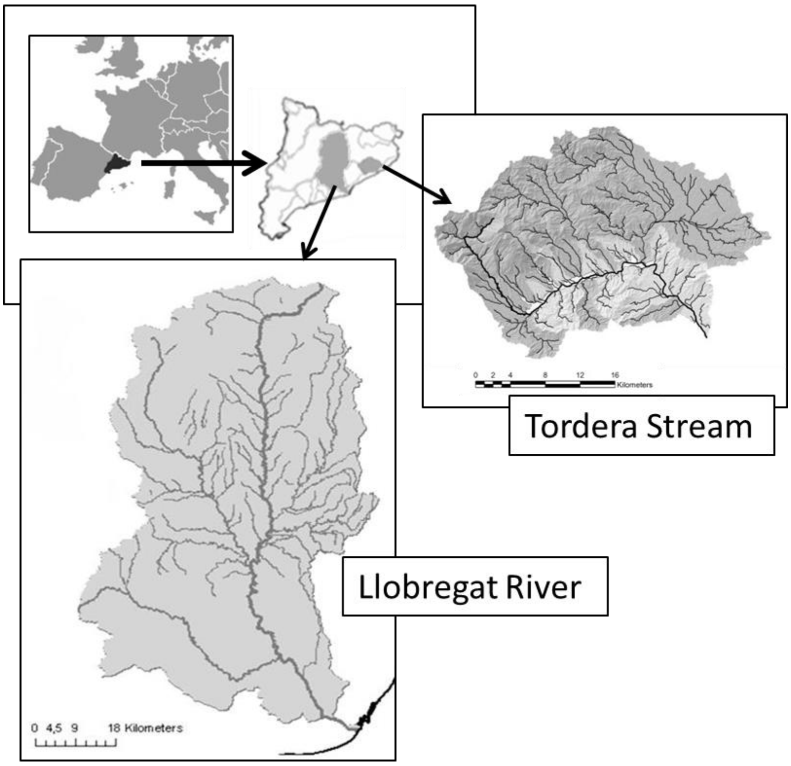


Fig. 1. Study areas encompassed in this thesis, including Llobregat and Tordera River Basins, in Catalonia, NE Spain.

The Llobregat

The Llobregat River rises in the Serra del Cadí (Pre-Pyrenees mountain ranges) at 1259 m a.s.l. and flows into the Mediterranean Sea after 156 km, with an average total annual discharge of 700 Hm^3 and 4,948 km^2 of drainage area. Discharge is about 30-50 $m^3\ s^{-1}$ in spring and about 2-20 $m^3\ s^{-1}$ in summer (weekly means). Low summer flows are also due to the severe abstraction of both surface and ground water (Ferrer, 1994; Custodio, 2012; Munné et al., 2012).

The Llobregat is a clear example of a Mediterranean river affected by multiple stresses over the last decades (Muñoz et al., 2012). It is regulated by one large dam on the mainstem and two others on its largest tributary (Cardener River), as well as by many small weirs scattered along the river course. This river basin is one of the main water supplies for the densely inhabited urban, agricultural and industrial area of Barcelona. Wastewater constitutes a significant input in the river, especially during periods of water scarcity. In the last 25 years, up to 63 wastewater treatment plants have been set up in the Llobregat basin to improve water quality (Munné et al., 2012). Ammonia concentration and nutrient load have decreased after the sanitation plan of the basin, but other contaminants continue to affect the lower course of the river (Munné et al., 2012; Petrovic and Barceló, 2012). Due to the diverse sources of pollution, a broad spectrum of hazardous organic and inorganic contaminants can be found at high levels in the river, often not accomplishing the Environmental Quality Standards (EQS) required by the European Directive 2008/105/EC (Munné et al., 2012). Trace metals are known from previous studies to occur in relatively high concentrations in water, sediment, and biofilms of the middle and lower river courses (Guasch et al., 2010; Sabater et al., 2012). In particular, the load of nickel in water was found higher than EQS concentration. The presence of potash mines that deposit mineral wastes in the Cardener sub-catchment results in a high concentration of sodium, chlorides and bromides downstream due to lixiviation and runoff (Prat and Rieradevall, 2006; Munné et al., 2012).

Fig. 2. The Llobregat River in Martorell (photo: Wikimedia Commons)

The Tordera

The Tordera Stream rises in the Montseny Mountains (Catalan Pre-Coastal Range) at about 1500 m a.s.l. and drains an area of *ca*. 895 km^2. Mean annual water yield is 170.4 million m^3 year^{-1} and mean discharge is *ca*. 4 m^3 s^{-1} (ACA, 2002). Mean annual rainfall in the basin ranges from 1000 mm near the headwaters to 600 mm on the coast (ACA, 2002; Rovira & Batalla 2006). There are no large dams along the stream. Land use in the Tordera catchment is agricultural, residential and industrial, leading to high nutrient loads in the middle and low course of the stream (Prat et al., 2002; Merseburger et al., 2005). Although industrial and urban pollution were very important until the early 1990s, the installation of wastewater treatment plants has improved water quality the basin in the last decades (Prat et al., 2002). The effects of low summer flows have been exacerbated by the widespread existence of legal and illegal water diversions for irrigation and domestic use (about 34% of water yield; Catalan Water Agency, 2002). This has lead the river to extensive dry beds (Fig. 3) affecting many kilometers of the stream in the upper and lower river course as well as the decrease of the aquifer level (Benejam et al., 2010).

The fish assemblage is composed of both native and non-native species (Benejam et al., 2008; 2010). The headwater reaches of both Tordera and Arbúcies, one of its main tributaries, are dominated by stocked brown trout *Salmo trutta*, whereas in the middle and lower reaches, native Mediterranean barbel *Barbus meridionalis* and Catalan chub *Squalius laietanus* are prevalent, coexisting with eel *Anguilla anguilla* and introduced Adour minnow *Phoxinus bigerri.* Grey mullets *Liza* sp. and non-native eastern mosquitofish *Gambusia holbrooki* are common in the lowermost reaches. Other species were reported from the watershed, like non-native common carp *Cyprinus carpio*, largemouth bass *Micropterus salmoides*, pumpkinseed *Lepomis gibbosus* and rainbow trout *Onchorhynchus mykiss.*

Fig. 3. The Tordera Stream at the same two sites near Sant Esteve de Palautordera, in spring (left) and summer (right).

Fig. 4. Fish species studied in the Tordera: Mediterranean barbel *Barbus meridionalis* (top) and Catalan chub *Squalius laietanus* (bottom).

Studied species

Chapters 3 and 4 are focused on the two native cyprinids of the Tordera stream, the Mediterranean barbel and the Catalan chub (Fig. 4), both endemic of north-eastern Spain and southern France, whose conservation status must be considered as “Vulnerable” according to IUCN criteria (Doadrio et al., 2011). Ecological aspects of the species investigated in the trace metal analysis are described in chapter 1.

The Mediterranean barbel *Barbus meridionalis*, endemic to the basins from Besòs (NE Spain) to Var (SE France) (Kottelat and Freyhof, 2007; Doadrio et al., 2011), is one of the smallest European species of the *Barbus* genus, attaining 270 mm (usually less than 200 mm) as a maximum length. Like most congeneric species, it is generally regarded as rheophilic and feeds mainly on benthic invertebrates. Reproductive period may vary among years from March to July, but usually takes place between May and June (Casals-Martí, 2005; Doadrio et al., 2011). Many populations of this species are threatened by several kinds of habitat loss (e.g. like river channelization), loss of river continuity (e.g. building of dams and weirs), water diversion, pollution and introduction of piscivorous nonnative species.

The Catalan chub *Squalius laietanus*, described in 2007 by Doadrio, Kottelat and De Sostoa as a separate species from *Squalius cephalus*, is a medium-sized cyprinid, up to over 400 mm standard length (García-Berthou and Moreno-Amich, 2000) but is usually smaller in the Tordera basin. It is spread from the Ebro basin to Portbou in Spain and from Agly to Tech Rivers in France (Doadrio et al., 2011). It is an omnivorous water-column dweller, found both in lentic and lotic waters. In streams and rivers, it usually inhabits run and pool mesohabitat. In Catalonia, spawning takes place between April and May, occasionally until July (Casals-Martí, 2005; Doadrio et al., 2011). The populations of this species are also contracting, due to pollution, wildfires and hydrological alteration (Doadrio et al., 2011).

Data collection

Fish sampling

For all of the three studies regarding fish, a four-person crew carried out one-pass electrofishing (Smith-Root LR-24 backpack electrofisher, 100-150 V, 0.8-1.5 A, fully rectified triphasic DC) in a 100-m reach for each sampling site: one person operating the anode, two people netting and one bringing a bucket. In the Tordera, fyke nets were also used as a second capture gear, as described in chapter 4.

In the Llobregat, the sampling was carried out in August 2010. All captured fish were preserved on ice and were frozen at -20ºC once in the laboratory. In the Tordera, we sampled every two months from May 2012 until June 2013. Captured fish were anesthetized with 5-10 drops of clove oil (Aura Cacia® pure essential oil) diluted in 10 L of water, measured to the nearest mm (fork length) and weighed to the nearest 0.1 g. Scale samples (5-10 each) were collected for age estimation from the area above the lateral line and posterior to the dorsal fin. On the first sampling occasion in mid-May, fish larger than 69 mm received a 9-mm Passive Integrated Transponder (PIT) tag (Biomark®) inserted with a pistol implanter into the body cavity, just posterior to the pectoral fin tip. In the following sessions, individuals longer than 69 mm were scanned for the presence of PIT tags. All unmarked fish were tagged during the study period, until all PIT tags available were used. Individuals shorter than 70 mm of both species were not tagged. Captured fish were released in the middle of the sampling reach following complete recovery.

Temperature and flow data

Water temperature was measured hourly at nine sites along the Tordera Stream and its tributary Arbúcies using data loggers (HOBO Water Temp Pro v2, Onset Computer Cooperation, Boume, MA). Water flow was also measured hourly with calibrated data loggers (Heron Instruments dipperLog and Solinst® 3001 Levelogger® Edge, Ontario, Canada) at one site impacted by water diversion and a control site, both located in the upper stream course. Flow data of the middle Tordera course were provided on a daily basis by the Catalan Water Agency (data available at http://www.gencat.cat/aca).

The statistical tools used in each study of this thesis are summarized in Table 1. Further details about methodology are described in each chapter.

Fig. 5. Field work on the Tordera Stream. Clockwise: electrofishing; measuring and weighting a chub; tagging a barbel with a PIT-tag gun; checking for marks with a PIT-tag reader; releasing tagged fish in the site of capture.

Table 1. Statistical analysis performed for each chapter of this thesis.

	Statistical tool	**Purpose of the analysis**
Chapter 1	Pearson's correlation	Relationship between fish size and trace metal concentrations
	Analysis of covariance	Variation of trace metal concentrations across sites and fish species, controlling for fish size
Chapter 2	Generalized linear models	Variation of water temperature and flow across sites and months
	Linear regression	Air - water temperature
	Analysis of covariance	Comparing air - water temperature relationship slopes among sites
	Circular statistics (Zar, 2010)	Calculating time of the day with minimum/maximum water temperature
	Whatson-Wheeler test	Comparing time of minimum/maximum temperature across sites
Chapter 3	Bowker's test for symmetry (Hoenig et al., 1995)	Checking for systematic bias between two different estimations of fish age
	Analysis of covariance, Mixed-effects linear models (Weisberg, 2010)	Variation of fish growth rates and body condition across sampling sites
	Minimum adequate linear/quadratic models	Checking effects of drought on fish growth and condition
Chapter 4	Mark-recapture models (Huggins, Cormack-Jolly-Seber, Robust Design)	Estimating fish abundance and survival probability

CHAPTER 1

TRACE METAL CONCENTRATION AND FISH SIZE: VARIATION AMONG FISH SPECIES IN A MEDITERRANEAN RIVER

Merciai R., Guasch H., Kumar A., Sabater S. & García-Berthou E. (2014). Trace metal concentration and fish size: variation among fish species in a Mediterranean river. *Ecotoxicology and environmental safety*, 107, 154-161.

Abstract

Concentration of trace metals (Al, Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb and As) in the muscle of six fish species was analyzed to determine the variation with fish size and fish species in an Iberian river with moderate metal pollution. Al, Fe and Zn were the most abundant metals across sites. Fish size and sampling site explained more variation than fish species, and a high intraspecific variability (among individuals) in metal loads was also observed. Considering the most spread species, concentrations were highest in bleak (*Alburnus alburnus*) and lowest in gudgeon (*Gobio occitaniae*) for all the elements. Metal loads were comparable with literature data from contaminated sites, often exceeding recommended European Environmental Quality Standards. The relationships between metal concentration and fish size varied markedly among sites, elements, and fish species. The slopes of these relationships were often significantly heterogeneous, a fact barely acknowledged in the literature, and were often negative, probably due to size-specific metabolic rates related to fish growth.

1. Background

Trace metals in aquatic environments may be of natural origin from rocks and soil or from human activities, e.g. industry, urban and agricultural discharge, mine runoff, solid waste disposal and atmospheric deposition (Bradl, 2005). Unlike other pollutants of organic origin, metals are not degraded or eliminated from the ecosystem (Rajkowska and Protasowicki, 2013) and accumulate in sediments and organisms (Zoumis et al., 2001; Ikem et al., 2003; Mendil and Uluozlu, 2007). Certain metals like manganese, zinc, copper, iron and nickel are essential (i.e. elements with recognized role in biological systems) in low concentrations for the aquatic organisms metabolism (Clark, 2001). Others, such as cadmium, lead and arsenic, are among the most toxic nonessential metals (Goyer et al., 1995; Canli and Atli, 2003). Even essential metals may be toxic for biological activities of organisms at certain concentrations (Pérez-Cid et al., 2001; Kucuksezgin et al., 2006).

Fish normally occupy high positions in aquatic trophic webs, accumulating several kinds of contaminants in their tissues, including trace elements (Bervoets and Blust, 2003; Noël et al., 2013). Heavy metals in fish represent a potential risk, not only to the fish themselves but also to piscivorous birds, mammals and even humans (Grimanis et al., 1978; Adams et al., 1992). Metal concentrations measured in fish are directly or indirectly influenced by a large set of abiotic and biotic factors. Fish can uptake trace metals by two main routes (Farkas et al., 2003; Terra et al., 2008; Rozon-Ramilo et al., 2011), either by adsorption from water through the gills, and from food absorbed through the digestive tract. The predominant pathways for heavy metal uptake appear to be highly variable over the range of metals, fish species and levels of contamination. Dietborne uptake is generally believed to be predominant in low-contaminated waters (Williams & Giesy, 1978; Dallinger et al., 1987; Farkas et al., 2003). The bioavailability, and so the bioaccumulation of trace metals in fish, depends thus on the concentrations in water and the rest of the ecosystem, e.g. algae, invertebrates and sediment, the latter being ingested with food by bottom feeders. Nevertheless, direct proportionality does not necessarily exist between water concentrations and bioaccumulation levels in aquatic organisms (Andres et al., 2000; Yi and Zhang, 2012).

Metals present in water show different bioavailabilities, both for fish and their prey. Water chemistry features such as dissolved and suspended organic carbon, pH, hardness and alkalinity are important modifiers of metal bioavailability and toxicity to aquatic organisms (Paquin et al., 2001; Niyogi and Wood, 2004). In addition, metals present in water show different bioavailabilities depending on whether they are in dissolved phase or bound to suspended matter (Bryan and Langston, 1992). Feeding behavior, habitat, fish size and age, sex, physiological conditions, spawning status or migration, even in the same area, can affect bioaccumulation beyond environmental concentrations (Andres et al., 2000; Canli and Atli, 2003; Farkas et al., 2003). Body size, in particular, has been shown to have a potentially strong effect on concentrations in muscle and other fish tissues and organs (e.g. Canli and Atli, 2003; Farkas et al., 2003; Yi et al., 2012; Noël et al., 2013).

We analysed the relative contribution of fish size and species, on muscle trace metal concentrations, in order to test whether the concentration-fish size relationship was consistent among sites and species. Negative relationships between fish size and muscle metal burden have been described (e.g. Canli and Atli, 2003; Farkas et al., 2003; McKinley et al., 2012) even though these relationships may be affected by the seasonal variation of fat content in the fish muscle (Farkas et al., 2003). We hypothesized that the environmental concentrations of heavy metals would affect the heavy metal concentrations in fish tissues. Since differences in heavy metal concentration between sites were low (Table 1), similar muscle loads were expected for each species along the river course, particularly for essential metals, whose excretion should be more efficient. We also expected higher concentrations of heavy metals to occur in bottom-feeding fishes, which often ingest sediments with their food.

1. Materials and methods

2.1. Study area and fish sampling

This study was undertaken to determine the trace metal contamination in fish from the Llobregat River, a good example of human-impacted watercourse, characterized by Mediterranean regime, regulated by three large dams located in its upper basin (see Methodology section for more details). Contaminants of urban, industrial and agricultural origin occur in the lower course of the Llobregat, when the river flows through the Barcelona conurbation. While being characterized by moderate metal pollution in all sites investigated, a slight upstream-downstream heavy-metal pollution gradient was found, based on water and sediment concentrations (Table 1). Aluminium, arsenic, cadmium, cobalt, copper, iron, lead, manganese, nickel and zinc were the target of our research because of their extensive presence in the Llobregat (Guasch et al., 2010). The sampling sites (Fig. 1) have been studied previously (e.g. Muñoz et al., 2009; Ginebreda et al., 2010; Ricart et al., 2010; Sabater et al., 2012). Site LL1 (Pont de Vilomara) is the least contaminated, situated upstream of the confluence with the Cardener river; site LL2 (Castellbell i el Vilar) receives some industrial effluents and surface runoff from agricultural areas; sites LL3 (Abrera) and LL4 (Martorell) are located in a densely inhabited area and receive urban and industrial wastewater inputs. In August 2010 a 100-m stretch in each site was sampled by single-pass electrofishing. All the fish captured were preserved on ice and were frozen at -20ºC once in the laboratory.

Table 1. Trace metal concentrations in water (μg l^{-1}), sediments (mg kg^{-1}) and fish (mg kg^{-1}) of Llobregat River. The data shown are average monthly observations for water concentrations (2003-2011) and average yearly observations for sediments (2004-2011) and fish (2010-2011). Minimum and maximum values are displayed between parentheses. Data provided by the Catalan Water Agency.

		Al	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Pb
Water	LL1	84.75 (30-520)	13.47 (2.5-55)	30.85 (10-230)	2.695 (0.5-5)	6.158 (2.5-10)	4.305 (1.5-13)	20.61 (6-76)	2.341 (2-6)	1.4 (0.3-2.5)	5.244 (5-10)
	LL2	84.87 (30-390)	15.34 (2.5-41)	30 (10-210)	2.775 (0.5-5)	6.4 (2.5-10)	4.3 (1.5-15)	23.07 (9-65)	2.346 (2-6)	1.405 (0.3-2.5)	5.375 (5-10)
	LL3	117.7 (30-700)	40.94 (2.5-144)	32.25 (10-250)	2.8 (0.5-5)	6.25 (2.5-10)	4.425 (1.5-10)	31.85 (10-102)	2.295 (2-4)	1.4 (0.3-2.5)	5.375 (5-10)
	LL4	120.8 (50-354)	50.3 (20-110)	70.25 (30-210)	11 (5-12.5)	11 (4-18)	5.98 (4.5-8.7)	23.5 (22-91)	2.5 (2-3)	0.7 (0.25-2.5)	1.8 (0.5-5)
Sediments	LL1	25979 (16149-38748)	401.4 (322.7-475.3)	19790 (16478-24554)	8 (7.1-9.8)	22.06 (16.6-27.5)	31.03 (20.5-42.6)	99.07 (73-125.1)	10.01 (7.3-15.1)	1.2 (0.4-1.8)	21.3 (17.6-27.4)
	LL3	29879 (15656-46763)	559.4 (404.3-920.8)	24518 (16482-39685)	10.03 (6.5-16.3)	26.34 (16.3-46.7)	35.1 (22.4-71.4)	105.3 (66-205.7)	14.2 (8.7-22.8)	1.371 (0.5-3.3)	25.9 (16.1-51)
Fish	LL1	233.8 (3.24-753.7)	0.324 (0.137-0.5)	19.91 (4-46.59)	0.076 (0.005-0.25)	0.242 (0.062-0.679)	0.785 (0.427-1.5)	9.586 (3.301-12.61)	0.092 (0.04-0.25)	0.0275 (0.0025-0.1)	0.19 (0.03-0.43)

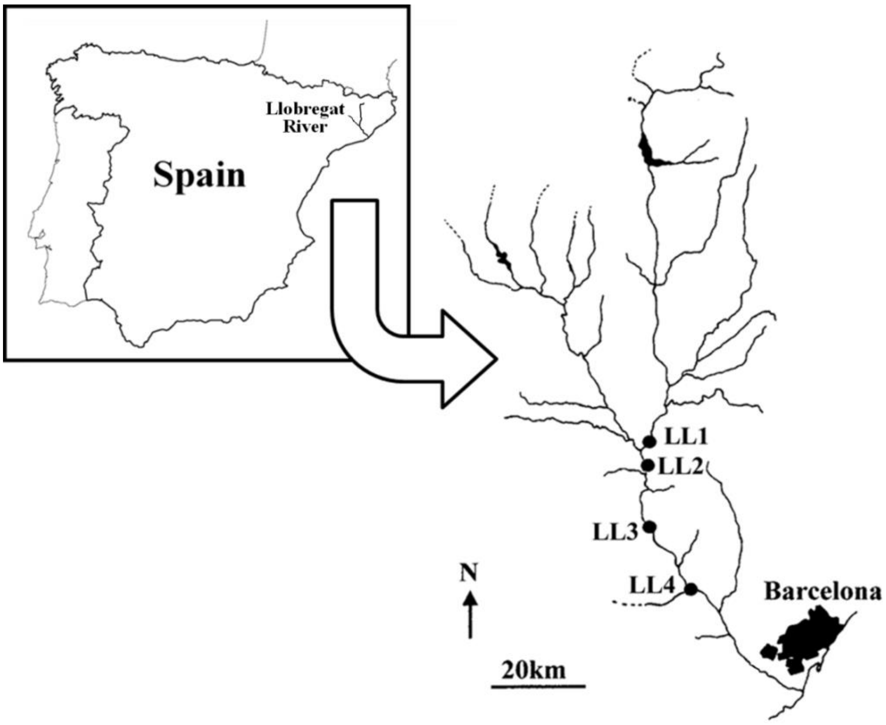


Fig. 1. Study area and sampling sites (LL1 to LL4) in the Llobregat River basin, NE Spain (see text for further details).

2.2. Sample preparation and analysis

In the laboratory, fish were determined by species, measured (fork length to the nearest mm), weighed (to the nearest *0.1* g) and then dissected. Filleted and skinned mid-dorsal muscle samples (1-5 g) were taken using stainless-steel tools, then lyophilised and weighed to determine dry weight (DW). Then, *ca.* 200 mg of DW were put in 4 ml of concentrated HNO_3 (65%, suprapure) and 1 ml of H_2O_2 (31%, suprapure) and digested in a high performance microwave digestion unit (Milestone, Ethos Sel). The microwave program followed four steps at temperature increasing from 85 to 210 ºC. The digested samples were then diluted with milli-Q water until 10 ml and treated as dissolved metal water samples. Metal concentrations were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS 7500 Agilent Technologies, Inc. Wilmington, DE), with the following detection limits in mg kg^{-1}: Al, 0.2937; As, 0.003; Cd, 0.007; Co, 0.004; Cu, 0.616; Fe, 4.044; Pb, 0.013; Mn, 0.167; Ni, 0.015; Zn, 0.3399. The accuracy of analytical methods was checked using certified reference materials (CRM 414, Community Bureau of Reference (BCR), Brussels, Belgium). Blanks with no fish tissue were run with each batch of samples to monitor contamination of used reagents. Muscle was chosen as a target-tissue since European Union (EU) limits are fixed to heavy metal concentrations in fish muscle and Spanish water agencies in general consider whole-fish or muscle loads, which are the most relevant in a fish consumption context. In order to make the results relevant for managers and comparable, we focused on muscle concentrations. Tissue metal burdens found in DW were converted to wet weight (WW) by multiplying by a factor of 0.2 (considering an average water content in fish tissues of 80%) (Shinn et al., 2009), with the aim of comparing Cd and Pb concentrations with EU limits.

2.3. Statistical analyses

Metal concentrations, as well as fish length, showed a right-skewed, non-normal distribution. Log_{10}-transformation was necessary to satisfy the assumptions of parametric statistical methods (normality, homoscedasticity and linearity). In particular, transforming both metal concentrations and fish length allowed obtaining the maximum linearity and the highest r^2 values in the relationships among the pairs of variables. The same was not the case with no transformation or transforming only metal concentrations. The relationship between fish size and trace metal concentrations (both log-transformed) were then first analysed with Pearson's correlation coefficient to test for possible relationships. Variation of heavy metal concentrations among species and sites was then analysed with analysis of covariance (ANCOVA), separately for each element. The model tested differences among fish species and sampling sites (both as categorical factors) with fork length as a covariate. We first tested for interactions among the covariate and categorical factors: if these are significant, they indicate that the slopes are not homogeneous and so the parallelism assumption of the standard ANCOVA is not satisfied, but they also imply effects of the terms involved, even if the factors alone are not significant (García-Berthou and Moreno-Amich, 1993). When the covariate was non-significant ($P > 0.10$ in this case), it was removed from the model to improve statistical power and a standard ANCOVA design was used (i.e. homogeneous slopes were assumed). All statistical analyses were performed with SPSS 15.

2. Results

An order-of-magnitude difference was found in metal concentrations from LL1-LL4 and LL2-LL3 sites of Llobregat River (Fig. 2). Table 1 summarizes the available historical metal concentrations data in the study area (data from the Catalan Water Agency). Average monthly observations are shown for water loads (2003-2011) and average yearly observations for sediments (2004-2011) and fish (2010-2011). Heavy metal concentrations of Mn, Co, Zn and As in water, and for all elements but Al and Cd in sediments increased significantly downstream (Spearman's correlations between site position and metal loads, all $P < 0.05$), according to the pollution gradient in these sites. A total of 188 barbel, 145 gudgeon, 46 bleak, 8 pumpkinseed, 5 roach and 4 carp were analysed. The average fork length and total weight of the fish analysed are shown in Table 2, while the average heavy metal concentrations in fish muscle DW for each species is shown in Table 3. Average element concentration of heavy metals in muscle displayed the same order in all species: Fe > Zn > Al > Mn > Cu > Ni > As > Pb > Co > Cd. The only exception was carp, in which Cu had the third highest concentration. Intraspecific variation among fish was remarkable. For instance, Ebro barbel showed up to three orders of magnitude between maximum and minimum individual Al concentration (Table 3). Among the three most abundant species, bleak showed the highest average concentrations of all analysed elements, and gudgeon the lowest.

Fish size and metal concentrations in tissue were correlated after pooling data of the three most abundant species from all the sites (Table 2). Correlation was negative and mostly significant for all elements and all species. The interaction of fish size (covariate in ANCOVA, Table 4) with site and fish species was significant for several elements, particularly for Al, Fe, Ni, Pb and As indicating that the slopes of the relationships between metal concentrations and fish size varied among sites and species (and that the parallelism assumption of standard ANCOVA) could not be assumed. The variation explained by the model (R^2_{adj}) was highest for Mn, Co, Ni, Cu, As, Cd and Pb. Most metal concentrations significantly varied among sites and species (after accounting for fish size) both considering the interactions and the main effects of factors.

Table 2. Pearson correlation coefficients (*r*) and their *P* values (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$) for the relationships between muscle metal concentrations and fish fork length. Species with low *N* (4 common carp, 5 roach, and 8 pumpkinseed sunfish) were excluded from analyses. Metal concentrations and fork length were $\log_{10}$-transformed. Average fish fork length and total weight (minimum and maximum between parentheses) are also shown.

	Bleak (*Alburnus alburnus*) (*N* = 46)		Languedoc gudgeon (*Gobio occitaniae*) (*N* = 145)		Ebro barbel (*Luciobarbus graellsii*) (*N* = 188)	
Fork Length (mm)	76.1 (41-99)		79.7 (44-102)		122.6 (35-355)	
Total weight (g)	5 (0.5-11.6)		5.9 (0.9-11.1)		39.3 (0.6-649)	
	r	*P*	*r*	*P*	*r*	*P*
Al	-0.758	***	-0.394	***	-0.491	***
Mn	-0.376	*	-0.376	***	-0.149	*
Fe	-0.691	***	-0.361	***	-0.381	***
Co	-0.400	**	-0.444	***	-0.189	**
Ni	-0.549	***	-0.507	***	-0.172	*
Cu	-0.283	0.057	-0.436	***	-0.112	0.125
Zn	-0.712	***	-0.156	0.064	-0.716	***
As	-0.081	0.590	-0.219	**	0.011	0.882
Cd	-0.417	**	-0.453	***	-0.135	0.065
Pb	-0.521	***	-0.483	***	-0.121	0.098

Table 3. Heavy metal load (mg kg^{-1}) in the muscle of the six fish species found in the Llobregat River. Averages (minimum-maximum) from each site are shown.

		Al	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Pb
LL1	*A. alburnus*	25.16 (2.30-93.3)	2.752 (1.58-4.68)	58.98 (28.1-134)	0.055 (0.032-0.066)	0.351 (0.261-0.47)	2.619 (2.12-3.21)	67.78 (38.6-109)	0.147 (0.064-0.24)	0.011 (0.005-0.023)	0.096 (0.065-0.17)
	C. carpio	7.63 (7.63-7.63)	2.47 (2.47-2.47)	48.83 (48.83-48.83)	0.044 (0.044-0.044)	0.40 (0.40-0.40)	2.04 (2.04-2.04)	32.91 (32.91-32.91)	0.154 (0.154-0.154)	0.006 (0.006-0.006)	0.058 (0.058-0.058)
	G. occitaniae	19.91 (2.48-69.5)	2.579 (0.72-15.2)	53.27 (17.7-159)	0.042 (0.007-0.22)	0.391 (0.11-1.27)	1.799 (0.59-9.49)	49.47 (18-110)	0.137 (0.019-0.53)	0.006 (0.002-0.019)	0.084 (0.027-1.43)
	L. gibbosus	18.93 (9.9-25.7)	0.965 (0.65-1.44)	44.97 (33.5-64.2)	0.039 (0.028-0.056)	0.394 (0.29-0.53)	1.469 (0.9-2.01)	37.48 (24.1-53.2)	0.247 (0.21-0.27)	0.009 (0.005-0.014)	0.078 (0.072-0.08)
	L. graellsii	42.03 (2.54-237)	2.603 (1.19-5.85)	65.93 (23.4-211)	0.05 (0.021-0.12)	0.342 (0.13-1.24)	2.478 (1.17-3.74)	45.24 (20.9-82)	0.158 (0.068-0.35)	0.007 (0.002-0.022)	0.095 (0.037-0.38)
	R. rutilus	52.69 (17.4-117)	2.822 (1.52-5.82)	60.55 (30.6-95.8)	0.055 (0.034-0.07)	0.368 (0.27-0.51)	1.602 (1.27-2.11)	60.7 (33.1-92.5)	0.176 (0.16-0.22)	0.004 (0.003-0.005)	0.107 (0.049-0.25)
LL2	*A. alburnus*	72.74 (9.06-349)	65.33 (24.1-196)	140.8 (48.7-310)	1.38 (0.78-2.77)	9.765 (3.42-32.2)	32.88 (20.3-59.9)	85.34 (43-188)	3.444 (2.15-5.54)	0.124 (0.029-0.46)	2.718 (0.99-9.08)
	L. gibbosus	31.78 (18.1-57.1)	18.7 (17.5-22.1)	116.8 (93.7-158)	0.671 (0.55-0.86)	5.615 (3.86-10)	18.06 (15.4-22)	36 (32.1-40.6)	5.677 (2.98-7.78)	0.055 (0.024-0.14)	1.211 (0.83-2.21)
	L. graellsii	13.29 (0.25-55.1)	36.77 (3.45-98.9)	73.61 (24.3-122)	0.762 (0.069-2.13)	3.765 (0.39-7.51)	29.81 (2.98-60.2)	38.57 (12.1-90.1)	4.23 (0.5-6.52)	0.055 (0.006-0.13)	0.959 (0.55-2.04)
LL3	*A. alburnus*	36.04 (10.19-214)	47.71 (25.63-126)	132.5 (74.2-418)	1 (0.52-2.89)	6.344 (3.33-26.6)	31.17 (19.7-54.2)	62.56 (44.9-118)	4.283 (1.59-9.19)	0.108 (0.025-0.31)	2.144 (0.76-11)
	C. carpio	11.78 (10.9-12.5)	15.4 (13.9-16.2)	120.4 (120-121)	0.89 (0.78-1)	4.03 (3.67-4.45)	40.3 (20.8-62.4)	43.24 (27-62.6)	2.691 (2.49-2.9)	0.121 (0.045-0.21)	1.518 (1.24-1.81)
	G. occitaniae	103.9 (103.9-103.9)	99.69 (99.69-99.69)	173 (173-173)	1.472 (1.472-1.472)	15.68 (15.68-15.68)	44.47 (44.47-44.47)	78.07 (78.07-78.07)	6.47 (6.47-6.47)	0.208 (0.208-0.208)	5.79 (5.79-5.79)
	L. graellsii	34.93 (4.37-101)	59.57 (23.6-162)	116.5 (50.3-225)	1.284 (0.69-2.6)	7.846 (3.59-29.5)	44.11 (21.5-72.1)	80.26 (45.6-134)	4.851 (2.35-8.36)	0.094 (0.02-0.38)	1.909 (0.71-8.67)
LL4	*A. alburnus*	92.72 (5.26-295)	5.118 (1.95-12)	116.5 (41.9-242)	0.175 (0.084-0.38)	1.52 (0.53-3.4)	3.119 (2.01-5.2)	114.2 (71.4-185)	0.185 (0.097-0.46)	0.012 (0.003-0.02)	0.232 (0.034-0.47)
	C. carpio	34.86 (34.86-34.86)	2.12 (2.12-2.12)	59.41 (59.41-59.41)	0.152 (0.152-0.152)	0.706 (0.706-0.706)	3.024 (3.024-3.024)	56.14 (56.14-56.14)	0.217 (0.217-0.217)	0.009 (0.009-0.009)	0.068 (0.068-0.068)
	G. occitaniae	132.9 (29.1-237)	4.482 (2.02-6.94)	105.7 (56.6-155)	0.12 (0.11-0.13)	1.687 (0.68-2.69)	3.505 (3.37-3.64)	71.19 (58.6-83.8)	0.199 (0.13-0.27)	0.015 (0.013-0.017)	0.2 (0.072-0.33)
	L. graellsii	26.3 (5.96-133)	2.901 (1.05-10.5)	55.16 (5-153)	0.082 (0.009-0.23)	0.524 (0.134-3.26)	2.391 (1.13-6.68)	67.88 (2.85-132)	0.254 (0.101-1.2)	0.005 (0.001-0.021)	0.071 (0.034-0.38)

Table 4. ANCOVAs of the effects of species and site (as categorical factors) on heavy metal concentrations in fish muscle, with fork length as a covariate (as in Table 2, species with low *N* were excluded from the analysis). Metal concentrations and fork length were $\log_{10}$-transformed. When the interaction between the categorical factors and the covariate was non-significant (in this case, $P > 0.10$), they were removed from the model and a standard ANCOVA design is reported. Degrees of freedom (df) respectively were 1, 3, 2, 4, 3, 2, 4 and 328 in the former model (which tests homogeneity of slopes) and 1, 3, 2, 5, and 337 in the standard model (which assumes equal slopes) (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$).

	Al		Mn		Fe		Co		Ni		Cu		Zn		As		Cd		Pb	
Source	SS	*P*	SS	*P*	SS	*P*	SS	*P*	SS	*P*	SS	*P*	SS	*P*	SS	*P*	SS	*P*	SS	*P*
	$R^2_{adj} = 0.399$		$R^2_{adj} = 0.903$		$R^2_{adj} = 0.478$		$R^2_{adj} = 0.908$		$R^2_{adj} = 0.871$		$R^2_{adj} = 0.938$		$R^2_{adj} = 0.443$		$R^2_{adj} = 0.923$		$R^2_{adj} = 0.819$		$R^2_{adj} = 0.907$	
Fork length (FL)	2.507	***	2.623	***	0.551	***	2.412	***	0.054	0.234	1.197	***	3.148	***	0.159	*	3.159	***	0.259	**
Site	1.288	**	24.80	***	0.267	*	23.61	***	0.281	0.061	20.14	***	0.193	*	0.854	***	18.34	***	0.130	0.269
Species	1.565	***	0.107	0.199	0.350	***	0.148	0.104	0.306	*	0.244	**	0.275	**	0.212	*	0.054	0.604	0.401	**
Site × species	1.545	**	0.145	0.495	0.344	**	0.300	0.103	0.234	0.187	0.274	*	0.314	*	0.246	0.103	0.521	0.087	0.124	0.442
Site × FL	1.297	**	-	-	0.297	**	-	-	0.080	0.551	-	-	-	-	0.302	*	-	-	0.072	0.540
Species × FL	1.651	***	-	-	0.365	***	-	-	0.278	*	-	-	-	-	0.191	*	-	-	0.388	**
Site × species × FL	1.545	**	-	-	0.340	**	-	-	0.261	0.144	-	-	-	-	0.276	0.072	-	-	0.111	0.501
Error	28.39	-	11.08	-	7.913		10.97	-	12.39	-	6.288	-	8.147	-	10.39	-	18.07	-	10.85	-

The relationships between the concentrations of Mn, Cu and Pb in the tissue and fork length in the two most frequent species across sites (bleak and Ebro barbel) are shown in Fig. 2. The accumulation patterns among these elements were similar and representative of the other metals. The relationship with fish size was significant for all elements except for Ni, As and Pb. Slopes were negative and fish size in general explained about 50-80% of the variance of metal concentrations. In all four sites the Al, Fe and Zn muscle concentrations were high (Table 3), whereas Mn and Cu concentrations were remarkable in sites LL2 and LL3, where the strongest heavy-metal content was detected. LL4, in spite of its location in the lower reach of the Llobregat River, downstream of industrial towns, showed concentrations similar to those of LL1. Based on the samples analysed, the average concentrations of Pb in WW exceeded the maximum limit established by the EU for fish muscle destined to human consumption (0.30 mg kg^{-1} WW) (Regulation (EC) No. 1881/2006 and 629/2008) in *A. alburnus* from LL2 (mean ± SD: 0.544 ± 0.497 mg kg^{-1} WW) and in all species at site LL3 (*A. alburnus*, *L. graellsii*, *C. carpio*, *G. occitaniae*, respectively: 0.429 ± 0.538, 0.382 ± 0.393, 0.304 ± 0.057, 1.160 ± 0 mg kg^{-1} WW). The average concentrations of Cd were always below EU limits (0.05 mg kg^{-1} WW).

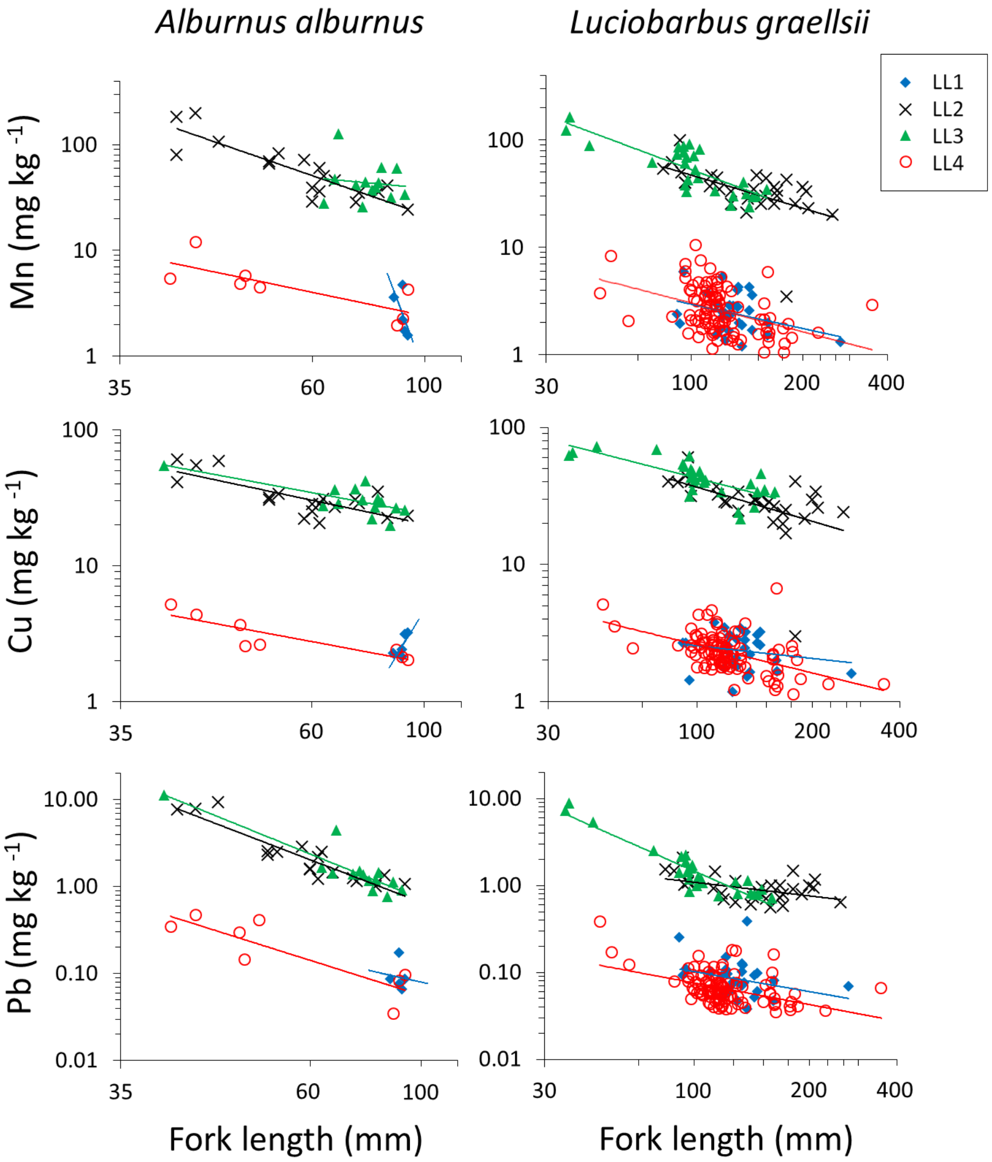


Fig. 2. Relationships of Mn, Cu, Pb concentrations with fork length of *A. alburnus* and *L. graellsii* (the species most frequent) by site (LL1 to LL4). Note the log scale of both axes. Regression lines by site are also shown.

4. Discussion

4.1 Relationships between metal concentrations and fish size

Muscle Pb concentrations in the Llobregat fish exceeded EU limits for several species, representing a potential risk for human consumption in case fish were eaten.

The concentration of all elements decreased significantly with fish size (as shown by the covariate or its interactions in the ANCOVAs). However, the response of many elements (Al, Fe, Ni, Pb, and marginally As) showed heterogeneous slopes and also variation with site and fish species. Therefore, the concentration of most heavy metals varied markedly with fish size, fish species and sampling site.

In contrast to our results, those observed in the literature do not often show significant relationship between metal concentrations and fish size or age. When significant, most relationships between fish and heavy metals have negative slopes, especially for two essential elements, Cu and Zn, and two non-essential, Cd and Pb (e.g. Al-Yousuf et al., 2000; Canli and Atli, 2003; Farkas et al., 2003; McKinley et al., 2012). The few cases with positive relationships regard Co, Zn, Cd and Pb (e.g. Łuczyńska and Brucka-Jastrzêbska, 2005; Monsefrad et al., 2012; Yi et al., 2012; Noël et al., 2013). Some elements showed different accumulation patterns in the same species from different studies, e.g. Cd in *Anguilla anguilla* (Barak and Mason, 1990; Noël et al., 2013). Farkas et al. (2003) found opposite trends for Pb in *Abramis brama* from the same site but in different seasons, as well as Cu and Zn concentrations, whose relationships with fish size were both non-significant in September and negative eight months later.

Several mechanisms can explain the predominance of negative slopes in the size-concentration relationships. This trend could be caused by tissue growing more rapidly than trace metal intake. As a consequence, seasonal fluctuations in body condition (weight-length relationship) could explain opposite trends in a given species sampled in different periods, suggesting a relative dilution effect of the lipid content of tissues, as hypothesized by Farkas et al. (2003). This assumption agrees with the fact that young fish have a lower percentage of fat tissue than adults (Shul'man, 1974; Weatherly and Gill, 1987; Braune et al., 1999). Furthermore, metabolic rate of organisms is size-specific and is higher in young specimens (Fagerstrom, 1977; Newman and Doubet, 1989), with consequent higher food intake and, in case of fish, the relative quantity of respiratory water passing through the gills per time unit. Both factors imply a higher potential uptake of trace metals in smaller than larger fish. The net accumulation of heavy metals in an organism is a result of the difference between uptake and depuration (Canli and Atli, 2003), the latter being an active process involving energetic costs. Since young fish invest relatively more energy in growth, less energy might be available for detoxification and this function might be more effective in large fish. Finally, other possible explications have been proposed, e.g. faster short-term uptake by smaller individuals, changes in body surface-volume ratio during growth, size-dependent feeding behavior, and size-dependent concentrations of some biochemical entity involved in accumulation kinetics, such as enzyme concentrations. Many of these factors are likely to interact (Newman and Mitz, 1988; Liang et al., 1999)

4.2. Variation among sites and species

The trace metal concentrations found were comparable with values from other studies in several species from contaminated freshwater ecosystems (Petkovšek et al., 2012; Noël et al., 2013). The maximum average concentrations corresponded to Al and the essential elements Mn, Fe, Cu and Zn. This observation may be related to the involvement of these elements in the regulation of key enzymatic detoxification processes, to extremely high uptake, or to disrupted metal metabolism (Goyer, 1997), since it is generally believed that fish actively regulate essential-element concentrations in tissues and that therefore tissue levels of Mn, Fe, Cu and Zn are influenced by several chemical and physiological processes in addition to environmental concentrations (Andres et al., 2000; Bervoets and Blust, 2003; Rejomon et al., 2010). Fish had higher burdens of trace metals in the intermediate sampling sites of the Llobregat River than in the most downstream one, which is known to be the most polluted; this pattern was likely due to lower bioavailability in the latter site.

Many factors can be at the origin of the differences between fish species collected in the same site, e.g. diet, lifespan, and physiological characteristics (Andres et al., 2000). Several authors attribute the interspecific differences observed in the metal burden of fish tissues to variation in diet (Liang et al., 1999; Canli and Atli, 2003; Rozon-Ramilo et al., 2011; Monikh et al., 2013). The three most common species in the present study show in general different feeding habits. Bleak is a water-column dweller, feeding on plankton, drifting invertebrates and terrestrial insects; Ebro barbel is an omnivorous bottom feeder; gudgeon is an invertivorous bottom feeder (Kottelat and Freyhof, 2007; Doadrio et al., 2011; García-Berthou et al., unpublished data). These feeding differences also imply different degree of contact with sediment, which represents a remarkable source of trace metals for aquatic organisms (Alagarsamy, 2006; Demirak et al., 2006; Noël et al., 2013). Bottom-dweller species are expected to accumulate a high load of metals (Barak and Mason, 1990; Johnson et al., 1998; Bolton et al., 2004). Our expectation that bleak would show lower metal loads than barbel, carp and gudgeon was not confirmed. However, Monikh et al. (2013) found discordant patterns of Cd, Co and Cu accumulation in fish species characterized by different degrees of association to sea bottom, concluding that the differences in metal concentration among the three fishes could considerably be attributed to the differences in feeding habits. Our results also suggest that the relationship of fish species with sediments is not central in the metal load variation among species. Bleak is an active and fast swimmer, whose consequently high metabolic rates could lead to concentrate metal loads higher than barbel and gudgeon (Serra et al., 1993; Kalay and Canli, 1999; Canli and Atli, 2003). Dietborne uptake only represents a fraction of total uptake, especially in highly contaminated waters (Farkas et al., 2003). Nevertheless, ascribing the variation among species to different rates of waterborne metal uptake would imply physiological differences and tolerance to physico-chemical stressors proper to every species.

In conclusion, fish size and heavy metal concentrations showed consistent negative relationships but that varied markedly among fish species, sampling sites and chemical elements. Many mechanisms can be involved in this variation, but size-specific metabolic rates, connected with growth, may contribute substantially. Metal concentrations across sites did not follow the pollution gradient observed in water and sediment, probably due to different bioavailabilities. Overall, our results suggest that heavy metal burden in fish varies strongly with size, species, site and chemical elements and further studies are needed to understand the mechanisms implied. Monitoring by water agencies should carefully consider the complex size-concentration relationships found in our study by selecting particular fish species and sizes or accounting for them in the analyses and management plans. In contrast to what could be expected with bioaccumulation, smaller fish can sometimes represent more hazards for wildlife and humans.

CHAPTER 2

SMALL WEIRS, BIG EFFECTS: DISRUPTION OF WATER TEMPERATURE REGIMES WITH HYDROLOGICAL ALTERATION IN A MEDITERRANEAN STREAM

Bae M.-J., Merciai R., Benejam L., Sabater S. & García-Berthou E. (2016). Small weirs, big effects: disruption of water temperature regimes with hydrological alteration in a Mediterranean stream. *River Research and Applications*, 32: 309–319.

Abstract

The effects of hydrological alterations on thermal regimes due to small water provisioning schemes are poorly understood. We studied the alteration of thermal regimes in a Mediterranean stream, where a weir and a water abstraction have been previously shown to severely affect the flow regime (e.g. frequency and duration of drought) and fish assemblage. Compared to non-impacted sites, the daily water temperature was more variable downstream of the weir, where water flow was reduced and drying occurred every summer. However, water temperature variation was smaller in a nearby downstream site dominated by effluents from a wastewater treatment plant. In addition, compared to all other sites, the times of the day to reach minimum and maximum water temperatures were markedly different in this site receiving the wastewater plant effluents and occurred earlier in the day in the site below the weir. The relationships between air and water temperatures were tight downstream but became looser and anomalous at the sites affected by water abstraction and effluent inputs. Overall, our results show that water temperature regimes in small streams are abruptly disrupted with water provisioning schemes with unknown consequences for aquatic organisms and ecosystems. Effects may be particularly stressful in Mediterranean-climate streams, where water is scarce and hydrological alterations pervasive.

1. Background

Water temperature is one of the most important physical factors in stream ecosystems because it has major influences on biogeochemical cycles, ecosystem functioning and life history and distribution of aquatic organisms (e.g. Allan and Castillo, 2007; Brown et al., 2007). Therefore, understanding the thermal regimes in stream ecosystems is fundamental for the ecological processes as well as for the management of freshwater resources (Poole and Berman, 2001; Dickson et al., 2012). In natural conditions, seasonal and daily variations of stream water temperature are influenced by local hydrological factors including stream discharge [turbulence and volume of water, and streambed influences (e.g. groundwater inputs and hyporheic exchanges), but particularly climate (e.g. air temperature, solar radiation, wind speed, precipitation and evaporation) and its spatial variation (e.g. latitude, altitude or riparian cover)] (Caissie, 2006). Above all, water temperature is closely related to air temperature (Smith and Lavis, 1975; Mackey and Berrie, 1991). As such, it might be expected that in natural conditions, mean daily water temperature tends to increase from upstream to downstream as well as from early morning to midday (Arscott et al., 2001; Caissie, 2006). Moreover, daily and seasonal water temperature variations are often lower in headwater streams (i.e. heavily canopied cover, light-limited systems) than in downstream reaches, which can be more influenced by meteorological conditions because of the open canopy and greater surface area (Johnson, 1971; Erickson and Stefan, 2000).

Anthropogenic disturbances such as regional land-use alteration (Malcolm et al., 2008), effluents from wastewater treatment plants (WWTPs), water releases from dams and water flow reduction due to impoundments and water diversions (Sinokrot and Gulliver, 2000) can alter the natural thermal regime in stream ecosystems. For instance, deforestation causes maximum water temperature to rise because of higher sunlight reaching the stream surface (e.g. Brown and Krygier, 1970). Impoundments generally decrease annual maximum temperature and temperature variation (Webb and Walling, 1993, 1996). In other cases, selective releases of cold hypolimnetic or warm epilimnetic water from reservoirs alter minimum and maximum water temperatures and reduce or increase the temperature downstream (Olden and Naiman, 2010). Furthermore, the continuous inflows from WWTPs may cause gradual increases of water temperature in the receiving streams. For instance, stream temperature has been shown to increase 0.11–0.21°C year-1 during winter and early spring (Kinouchi et al., 2007) and 3.8°C on average (Xin and Kinouchi, 2013) in waters downstream urban WWTPs in Japanese rivers.

Mediterranean streams are characterized by seasonally variable streamflow, strong variations of water temperature, and summer droughts (Acuña et al., 2004; Bonada and Resh, 2013). This variability causes shifts in water availability and properties across temporal and spatial scales. In these systems, flow regulation (e.g. reservoir and weir construction) has been one of the priorities associated to high water demand (García de Jalón et al., 1992). The anthropogenic disturbances due to water abstraction, weir construction and wastewater inputs have profoundly altered the natural thermal regimes in Mediterranean rivers. For example, Dolz et al. (1994) found a decrease in daily water temperature range as well as changes of daily water temperature patterns below large reservoirs in the Ebro River. Prats et al. (2010) reported that in the Ebro River the difference in monthly water temperature above and below reservoirs was ca 6°C and that mean water temperature increased 3°C immediately downstream a nuclear power plant. Todd et al. (2005) showed that daily water temperatures decreased up to 10–12°C downstream of the fourth largest dam in Australia. In addition, the effects of decreased summer temperatures extended into a hundred of kilometres in Murray–Darling River basin in Australia (Preece, 2004; Todd et al., 2005). Most of the described research on thermal regimes in regulated rivers has focused on large systems. There has been little attention to the changes of thermal regimes due to anthropogenic disturbances in small streams, even though they represent most of the drainage area and length of rivers (Allan and Castillo, 2007). Similar disturbances may produce very different consequences in small streams than in large rivers. For instance, deforestation would produce drastically higher thermal responses in small streams, causing abrupt changes in lotic communities (e.g. Brown and Krygier, 1970; Johnson, 2004). Also, damming may interfere water flow in a much larger proportion in smaller streams, with implied higher consequences for water temperature. Benejam et al. (2010) showed that the flow regime of several Mediterranean streams had lower flows and more frequent droughts than those corresponding to natural flow regime. In particular, one stream (Tordera stream) was severely affected by a weir, which abstracted water for irrigation and human consumption and produced artificial drying several weeks per year; more downstream, water flow is more permanent because of the inflows of WWTPs.

Therefore, we aimed to investigate the consequences that these hydrological alterations (i.e. water abstraction through a weir and water inputs from a wastewater treatment plant) may have for the thermal regime in a Mediterranean stream. Specifically, we aimed to assess the effects of hydrological alterations on the following: (1) the upstream–downstream gradient in seasonal and daily thermal regimes and (2) the air–water relationships. Understanding the effects of hydrological alteration on the air–water relationships is useful because of the relevance of air temperatures on stream water temperature. Lack of correlation between the two may be indicative of the effects on water temperatures.

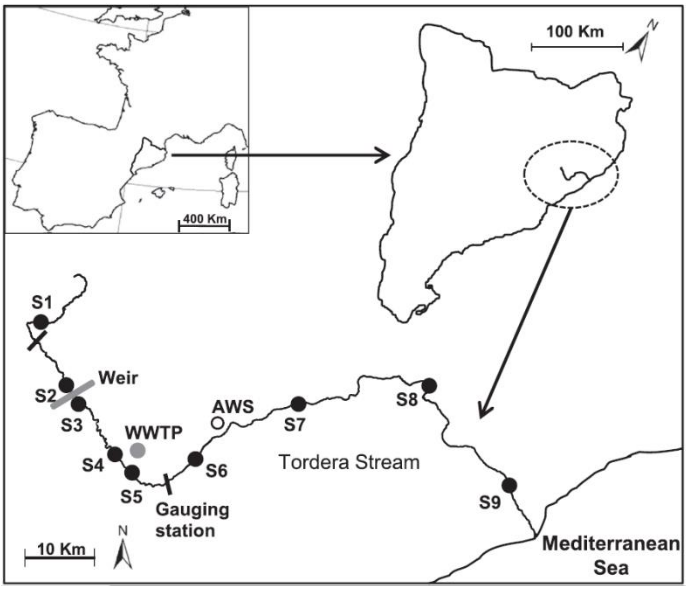


Fig. 1. Location and detailed map of the Tordera stream, showing the study sites where air and water temperatures and water flow were measured. AWS indicates automatic weather station and bars indicate two official gauging stations. See Table 1 for precise location of sampling sites.

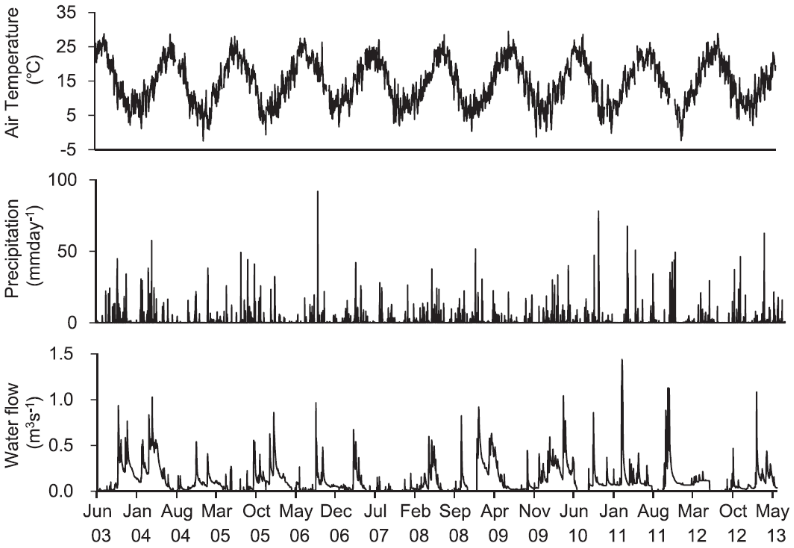


Figure 2. Temporal changes of daily air temperature, precipitation and water flow in site S6. Water flow data were log10(x+1) transformed. Labels in the x-axis indicate month (letters) and year (e.g. “03” = 2003).

1. Material and methods

2.1 Study sites

The study sites were located in the Tordera basin (see Methodology section for more details). Air temperature measurements near site S6 (Table 1) from 2003 to 2012 showed only 1.1 days per year of freezing air temperatures (≤ 0°C), being -2.5°C of minimum temperature 29.5°C of maximum temperature (Figure 2). Air temperature is much cooler in upstream reaches; in site S1, 89.0 days in a year (2005–2006) had freezing air temperatures, which ranged from -9.0°C to 25.7°C. Mean annual precipitation during the period (2003-2012) was 566.4 mm, and seasonal heavy rain mostly falls in spring (165.6 mm) and autumn (188.9 mm). The prolonged drought in summer decreases streamflow and increases stream drying (Benejam et al., 2008; Acuña et al., 2009; Mas-Martí et al., 2010). Upstream reaches (site S1; Figure 1) have little human activity and high percentage of forest (dominated by *Quercus* spp.). Row-crop agriculture and residential areas increase downstream in the basin. The mean annual water yield in the Tordera stream is ca 170.4 million m^3 and nearly 57.8 million m^3 are abstracted annually (ACA, 2002). Water flow is strongly influenced by a small weir and water abstraction in the middle (S3–S4) and lowermost (S9) reaches (Benejam et al., 2010). The weir is located between sites S2 and S3, and the reach between S3 and S4 dries almost every summer for several weeks (Benejam et al., 2010). Further, effluents from a WWTP (1300 m^3 day^{-1} of mean outflow) are released between sites S4 and S5 (Ortiz et al., 2006) and the effluents make the flow regime to be permanent downstream of WWTP (i.e. S5 to S8).

2.2 Hydrological data

Water and air temperatures and water flow were measured in the Tordera stream (Table 1). Water temperature was measured at nine sites along the Tordera stream on an hourly basis using data loggers (HOBO Water Temp Pro v2, Onset Computer Cooperation, Boume, MA). Air temperature measured on a daily basis was obtained from an official automatic weather station near site S6. Water flow was measured on an hourly basis with calibrated data loggers (Heron Instruments dipperLog and Solinst® 3001 Levelogger® Edge, Ontario, Canada) in sites S2 and S4 and provided on a daily basis for the three official gauging stations (near S1, S6 and S8) by the Catalan Water

Table 1. Location and features of the sampling sites and measuring periods. WT = Water temperature (hourly records), WF = water flow, AT = air temperature.

Sites	UTM X	UTM Y	Altitude (m a.s.l.)	Drainage area* (km^2)	Distance from source* (km)	Measuring variables	Measuring periods
S1	449261	4625070	522	15.11	5.746	WT	2009-10-7 to 2013-6-19
Official gauging station (Llavina, S1)	450000	4622691	365	48.75	9.644	WF	
S2	450862	4620754	305	56.49	12.27	WT, WF	2012-5-30 to 2013-6-19
S3	451946	4619585	267	58.89	14.88	WT	2009-10-20 to 2013-6-19
S4	454803	4615455	173	79.35	20.94	WT, WF	2009-10-7 to 2013-6-18
S5	455681	4614381	135	81.42	22.63	WT	2012-7-22 to 2013-6-17
Official meteorological station (Sant Celoni, near S6)	456838	4615063	170	-	-	AT	
Official gauging station (Sant Celoni, near S6)	457561	4114617	122	124.0	24.70	WF	
S6	459053	4615859	120	135.6	29.06	WT	2009-12-1 to 2013-6-17
S7	467054	4619832	67	283.1	39.67	WT	2012-4-17 to 2013-6-18
Official gauging station (Fogars de Tordera, near S7)	474643	4620999	37	766.4	49.90	WF	
S8	474643	4620999	37	766.4	49.90	WT	2009-12-1 to 2013-6-17
S9	481311	4611807	1	864.4	61.99	WT	2012-4-18 to 2013-4-6

[*] Data from Generalitat de Catalunya (http://www6.gencat.cat/mediamb/sig/cartografia/hidro_1_50000.html)

Agency (data available at http://www.gencat.cat/aca). Table 1 indicates the precise location of the measurement sites and the period of measurements. Study sites were considered to be in the drying phase when isolated pools were formed, the streambed completely dried out, or when superficial water flow of 0 $m^3 s^{-1}$ was reported. Long-term data was interrupted in some cases because of floods or vandalism; also, water temperature could not be measured when data loggers were outside of water because of floods or drying.

2.3 Data analysis

Four different methods were applied to compare temporal and longitudinal variability of water temperature. First, generalized linear models (GLM; McCullagh and Nelder, 1989) were used to test whether water flow, water temperature or daily water temperature variation differed with sampling month and site (categorical factors). We applied GLMs with gamma error and log link functions for water flow and normal errors and identity link functions for water temperature and daily water temperature variations. At first, we used all the nine study sites to compute GLMs, and subsequently we applied GLMs only for sites S3 and S4 (severely affected by the weir influence) and S4 and S5 (located downstream the WWTP) to specify the effects of anthropogenic disturbances to the changes of thermal regimes.

Second, the monthly mean daily temperature range was calculated based on hourly measured water temperature data. Only the data with more than 50% of records within each month were used for calculating mean daily water temperature range in order to avoid biases.

Third, two different linear regression analyses were conducted to figure out the effects of anthropogenic disturbances. First, linear regression between air and water temperature (daily minimum, mean and maximum, respectively) was applied to investigate whether the contribution of air temperature to water temperature changed from upstream to downstream, or according to anthropogenic disturbances. Then, we applied analysis of covariance in order to compare the differences of slopes amongst study sites. Second, linear regressions using daily mean, minimum and maximum and hourly based water temperature data were performed between two adjacent sites (measurements at the upper and lower sites as independent and dependent variables, respectively) to examine whether the influences of water temperature measured in the upper reaches were different according to the strength of anthropogenic disturbance (i.e. weir construction or effluents from a WWTP). GLMs and linear regression analyses were performed with SPSS 20. Analysis of covariance was calculated in the R statistical environment (R Core Team, 2013).

Lastly, for the time of the day needed to reach minimum and maximum water temperatures, we used circular statistics (Zar, 2010), because for instance 1:00 h is closer to 23:00 than to 4:00 contrary to what the numerical values suggest. We compared these times of the day amongst sites with the Watson-Wheeler test in the ‘circular’ package (Agostinelli and Lund, 2013) of the R statistical environment (R Core Team, 2013). The relative frequency (%) of the time was represented in a gradient map using Matlab 7.0.

2. Results

Water flow showed significant variations with month, site and also month × site, the latter indicating that its seasonal variation was different at different sites (Table 2). In general, water flow increased from upstream to downstream, but anomalies existed in the uppermost site (S1, higher flow) and in the site below the weir (S4, lower flow) (Figure 3). Water flow was the lowest in S4, ranging 0.09–0.95 $m^3 s^{-1}$ (average: 0.31 $m^3 s^{-1}$), and highest in S8 (downstream) (0.15–0.99 $m^3 s^{-1}$, average: 0.59 $m^3 s^{-1}$). Water flow was generally higher in wetter months (March to May), ranging from 0.51 to 1.05 $m^3 s^{-1}$, and strongly decreased during the dry season (July to August), ranging from 0.04 to 0.36 $m^3 s^{-1}$. Stream drying was accentuated downstream of the weir, especially in S4 (28.4%) (Figure 4), and mainly from summer to early autumn (July to October).

Monthly water temperature was different amongst months and sites, with also significant month × site interaction (Table 2, Figure 5). GLMs similar to those of Table 2 but only comparing S3 and S4 or S4 and S5 also showed significant differences in temperature amongst months, sites and month × site interaction, indicating changes amongst these three consecutive sites. Monthly water temperature increased from upstream (S1: 6.1–16.6°C) to downstream (S9: 9.6–24.3°C), and the differences increased in summer. In site S5, where effluents were continuously released from the WWTP, monthly water temperature was higher (21.5–22.2°C) in summer periods (July and August) than that in the adjacent sites (e.g. S4: 15.0–19.1°C and S6: 17.8–20.7° C). In spite of the weir between S2 and S3, the monthly water temperature was similar between S2 (5.9–19.6°C) and S3 (6.4–20.0°C). On the other hand, monthly water temperature was higher in site S4 (8.3–19.1°C) than in the upstream site S3, except in summer. Similarly, daily water temperature variation was significantly different amongst sites and sampling periods, either considering all sites (Table 2) or only comparing S3 with S4 or S4 with S5. Monthly mean daily temperature was lower in upstream sites such as S1 (1.0–2.6°C) and S3 (1.2–2.6°C), whereas the variation increased in sites S4 (1.6–6.2°C) and the most downstream site (S9, 0.3–7.1°C) (Figure 6). Overall, the daily variation was higher in April, when it ranged from 2.3°C (S1) to 6.2°C (S4), and lower in summer and autumn, except for S4. The daily variation in S4 was always higher than the adjacent sites during the whole study period. However, the daily variation in S5 was relatively minor regardless of the month, ranging from 1.2°C (November in 2012) to 3.8°C (April 2013).

The daily mean air temperature explained more than 70% of the variation in daily minimum, mean and maximum water temperature in all the study sites, except for maximum temperatures in S7, and the slopes varied significantly amongst sites (Table 3). The slope of the air–water temperature relationships for mean, maximum and minimum water temperatures was higher downstream (e.g. S9: 0.82–0.84) than upstream (e.g. S1: 0.55–0.56). The slope was the lowest (0.47–0.59) in S4 (the site downstream of the weir) and higher (0.70–0.79) in S5 (site affected by the inflow of effluents) than in adjacent sites.

The linear regression based on water temperatures between two adjacent study sites generally showed higher explanatory power (r^2: 0.76–0.97) and slope (0.65–1.33) (Table 4) compared with the contribution of air temperatures. The slope and explanatory power did not show much difference according to the measuring intervals (i.e. daily or hourly) and the slopes between two adjacent sites were close or greater than 1 (i.e. warmer temperatures downstream) in study sites less influenced by anthropogenic disturbances as well as between S2 and S3. However, the slopes between S3 and S4 (0.70–0.75) and between S5 and S6 (0.66–0.77) were lower.

The time to reach daily minimum and maximum water temperatures was significantly different amongst sites (minimum: Watson-Wheeler test, W = 910.2, d.f. = 16, $P < 0.001$ and maximum: W = 1614.5, d.f. = 16, $P < 0.001$) reflecting the effects of the weir and the effluents from WWTP. Daily minimum water temperatures generally occurred in the morning (around 9:00 to 10:00) but 2–3 h earlier in S4 (7:00, 35.0% of the days) (Figure 7). In the case of S5, the daily minimum water temperature occurred at about 14:00 (50.0%). Daily maximum temperature usually took place between 17:00 and 18:00 but was earlier in S4 than in other sites (15:00 and 16:00, 41.5 and 36.6%, respectively). The maximum water temperature in S5 was more variable but generally occurred at night time (i.e. 22:00–01:00).

Table 2. Generalized linear models (GLM) of water flow, water temperature, and daily water temperature variation with sampling month and site as factors. All Wald chi-square statistics were significant (all $P < 0.001$); d.f. = degrees of freedom; "Month2" indicates a factor with all months from October 2009 to May 2013. See Methods for details on GLMs.

Dependent variable	Effects	d.f.	Wald Chi-Square
Water flow	Month	11	861.3
	Sites	4	215.2
	Month × Sites	44	449.9
Water temperature	Month	11	33257.9
	Sites	8	8442.1
	Month × Sites	76	1224.2
Daily water temperature variation	Month2	42	3317.8
	Sites	8	3411.9
	Month2 × Sites	144	2633.9

Table 3. Summary of the linear regression between mean, maximum, and minimum water temperature and air temperature at nine sites. All the *P* values for slopes and intercepts were less than 0.01.

Water temperature statistic	Study site	Slope	Intercept	r^2
Mean	S1	0.55	2.71	0.82
	S2	0.69	1.67	0.88
	S3	0.67	2.33	0.88
	S4	0.52	5.54	0.76
	S5	0.73	3.76	0.87
	S6	0.62	5.34	0.88
	S7	0.65	6.53	0.81
	S8	0.71	5.38	0.88
	S9	0.84	3.60	0.87
Maximum	S1	0.56	3.53	0.83
	S2	0.69	2.62	0.89
	S3	0.71	2.93	0.87
	S4	0.59	6.59	0.79
	S5	0.79	4.21	0.87
	S6	0.64	6.82	0.87
	S7	0.71	8.18	0.66
	S8	0.80	6.14	0.85
	S9	0.82	5.95	0.82
Minimum	S1	0.55	2.04	0.79
	S2	0.68	0.91	0.87
	S3	0.64	1.67	0.86
	S4	0.47	4.68	0.71
	S5	0.70	3.33	0.84
	S6	0.61	4.16	0.82
	S7	0.62	4.94	0.77
	S8	0.67	4.42	0.85
	S9	0.84	2.06	0.86

Table 4. Summary of the linear regression between water (daily minimum, mean and maximum, and hourly basis) temperatures in two adjacent measuring sites. All the *P* values for slopes and intercepts were less than 0.01.

Water temperature statistic	Dependent variable	Independent variable	Slope	Intercept	r^2
Mean	S2	S1	1.20	-0.90	0.82
	S3	S2	1.04	-0.14	0.93
	S4	S3	0.72	4.09	0.88
	S5	S4	1.21	-1.90	0.94
	S6	S5	0.73	4.35	0.98
	S7	S6	1.14	-0.42	0.97
	S8	S7	0.90	-0.56	0.94
	S9	S8	1.15	-1.84	0.97
Maximum	S2	S1	1.16	-0.57	0.83
	S3	S2	1.11	-0.80	0.92
	S4	S3	0.75	4.79	0.88
	S5	S4	1.32	-4.23	0.94
	S6	S5	0.66	5.56	0.92
	S7	S6	1.35	3.03	0.82
	S8	S7	0.87	-4.24	0.82
	S9	S8	0.93	1.17	0.92
Minimum	S2	S1	1.21	-0.91	0.79
	S3	S2	1.01	0.15	0.92
	S4	S3	0.70	3.54	0.85
	S5	S4	1.09	-0.17	0.93
	S6	S5	0.77	3.50	0.95
	S7	S6	0.95	2.18	0.92
	S8	S7	0.87	2.29	0.94
	S9	S8	1.26	-2.88	0.96
Hourly	S2	S1	1.19	-0.78	0.81
	S3	S2	1.04	-0.10	0.92
	S4	S3	0.73	3.90	0.83
	S5	S4	1.10	-0.59	0.85
	S6	S5	0.73	4.36	0.95
	S7	S6	1.10	0.24	0.90
	S8	S7	1.10	-0.27	0.90
	S9	S8	1.07	-0.65	0.89

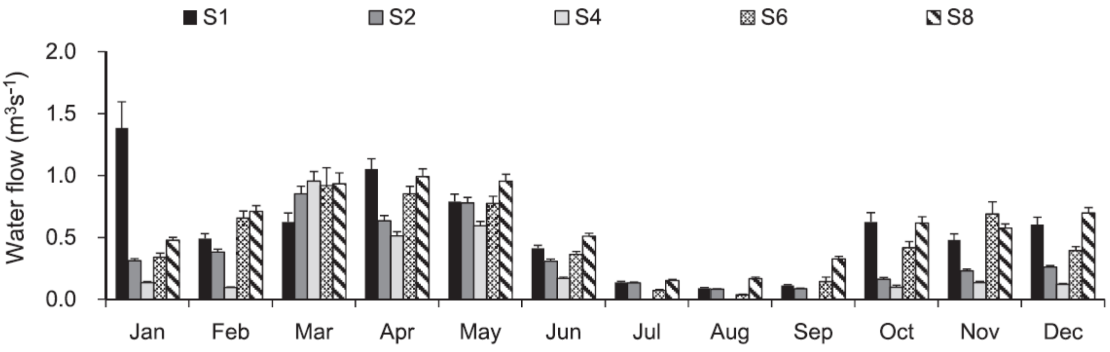


Fig. 3. Mean monthly discharge in the different sites from upstream (S1) to downstream (S8)

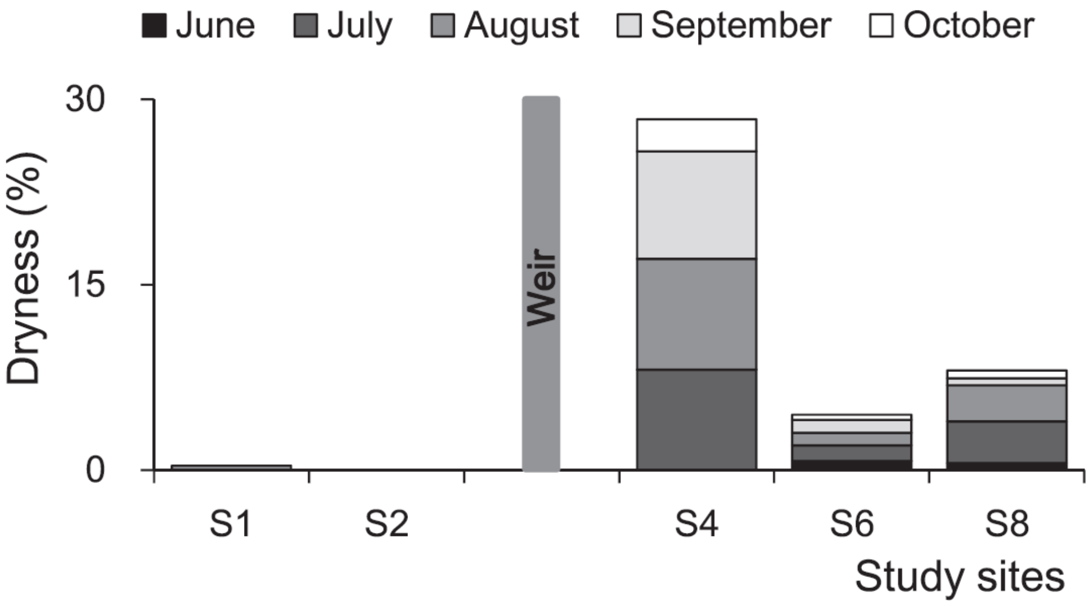


Fig. 4. Frequency of drying by month and site (above and below the weir). No site was dry the rest of months not shown.

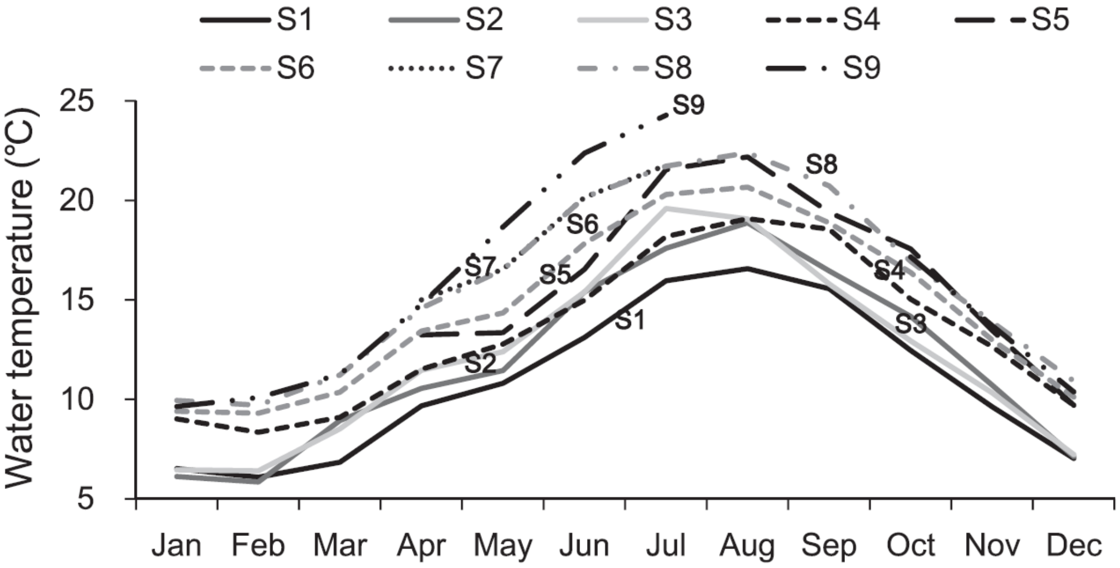


Fig. 5. Variation in monthly mean water temperature from upstream (S1) to downstream sites (S9).

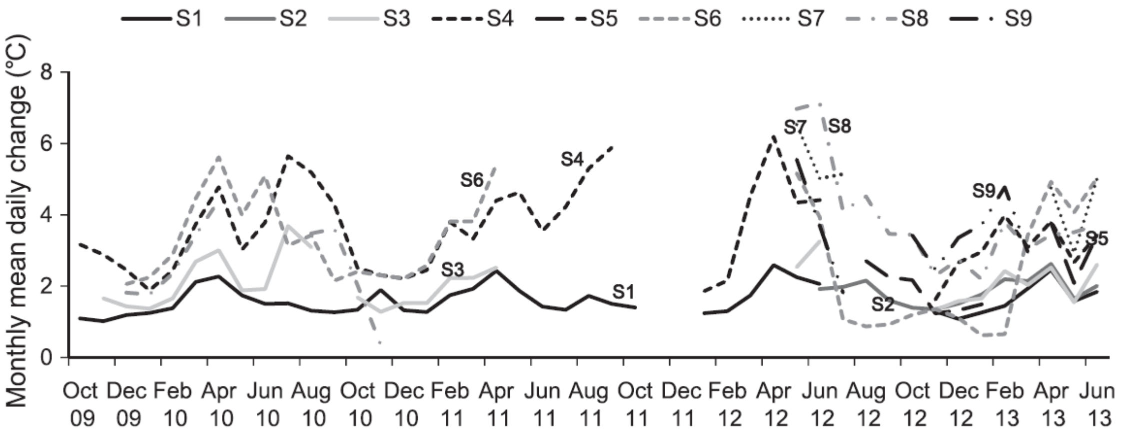


Fig. 6. Monthly mean daily temperature range at the different sites from upstream (S1) to downstream (S9).

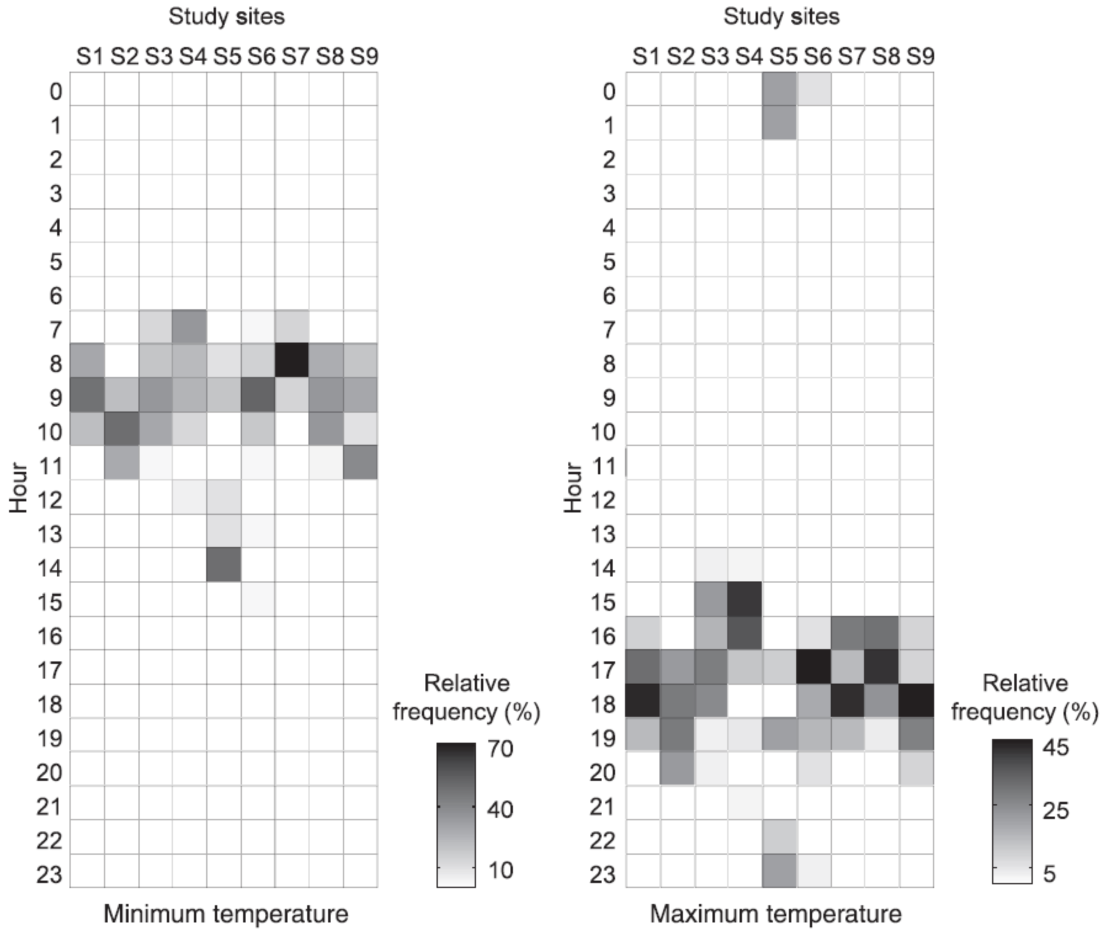


Fig. 7. Frequency of the different times of day (hour) to reach daily maximum and minimum water temperatures in the different sites (S1-S9). S4 is the site below the weir and S5 receives the effluents from a wastewater treatment plant.

3. Discussion

Clear thermal gradients were observed from upstream to downstream except in the sites with relevant anthropogenic disturbances. Similarly, water temperature in each study site also showed foreseeable seasonal and daily rhythms that generally depended on the heat energy gained or lost in the stream reach, the thermal inertia (e.g. water volume) and the main sources of runoff (Ward, 1985; Olden and Naiman, 2010). These patterns were, however, disrupted in the altered sites, for instance in the time of the day when minimum and maximum temperature occurred. Inflow of ground water and dense canopy played an important role to determine the thermal regime in the uppermost sites (e.g. S1), where anthropogenic disturbance was minimal. Dense canopy cover blocks the reaching of solar radiation into stream channel, decreases heat load and buffers the influence of changing weather conditions (e.g. wind) in nearstream (Li et al., 1994; Davies and Nelson, 1994). In addition, water temperature becomes stabilized as the ground water with constant temperature constantly enters the stream (Webb, 1992; Poole and Berman, 2001). Therefore, air temperature was less influential in determining stream temperature upstream, as highlighted by the lower slopes of linear regressions between air and water temperatures (Erickson and Stefan, 2000; Caissie, 2006) in that site. Furthermore, daily water temperature fluctuations upstream were usually lower than downstream, although the range and fluctuation of daily temperature varied seasonally. Usually, heat exchange is more rapid and directly influences water temperature downstream with wider channels and less riparian vegetation causing more rapid heat conduction and radiation. Open canopy in the riparian zone downstream makes solar radiation to directly penetrate into the stream channel (Poole and Berman, 2001; Caissie, 2006). In addition, stream water from nearby tributaries constantly flows and the groundwater is less influential downstream compared with upstream area (Webb, 1992). As such, even if weather conditions are similar from upstream to downstream, air temperature is more influential downstream, causing higher fluctuations of daily water temperature and steeper slopes in the relationships between air and water temperature (Webb, 1992; Caissie, 2006).

We found alterations in the thermal regime in the sites affected by a small weir used for water abstraction and by effluents from WWTP. This set of hydrological alterations is common in streams of the Mediterranean region (Bonada and Resh, 2013). The impact of the weir to the alterations of the thermal regimes was more distinct in S4 than in S3, the downstream site beyond the immediate vicinity of the weir. Even though the weir was located between S2 and S3, considering the linear regression results between two adjacent sites, the changes of water temperature were similar between S2 and S3 compared with other cases because of the short distance between those two study sites (ca 1.3 km) and the similar land use. Dense canopy cover in those two study sites (the relative forest ratio in S2 and S3: 87.0% and 83.7%) keeps the stream water evapotranspiration, resulting in similar monthly water temperature as well as water temperature variation in spite of the weir being between S2 and S3. However, the abrupt increase in agricultural area (36.9%) and low forest ratio (31.0%) increased the influence of the water abstraction from the weir in S4, where the main water source was the water from upstream.

Because both dam and weirs generally change the flow regime compared with natural conditions (i.e. before construction), the influence of solar radiation and air temperature and the response of stream to heat exchange can be altered. Furthermore, the potential flooding of the floodplain is reduced downstream of dams because of decreases in flood frequency (Erickson and Stefan, 2000). Weirs also reduce the water depth and flow downstream, causing habitat simplification (Kim et al., 2013). Moreover, the duration and frequency of stream drying downstream increase, with detrimental effects on native fish assemblages and other aquatic organisms (e.g. Bunn and Arthington, 2002; Benejam et al., 2010). Below large dams, the differences in temperature variation generally become smaller because cold water is generally released from the hypolimnion of reservoirs (Steel and Lange, 2007; Angilletta et al., 2008) and water temperature is generally decreased during spring and summer and increased during winter, compared with natural conditions (Olden and Naiman, 2010). We observed a similar pattern: the monthly temperature downstream of the weir (S4) was 1.94–2.75°C higher during autumn and winter, whereas during summer, the temperature was sometimes -0.40°C higher in S2 (upstream of the weir).

The relationship between air–water temperatures can be also altered by different types of anthropogenic disturbances. Webb (1992) reported that the explanatory power (R^2: 0.59–0.98) as well as the slope (i.e. 0.59–0.95) of linear regressions between air–water temperature tended to increase as a function of the distance from a large reservoir. A similar pattern was observed in our study by the influence of the weir. In that site, the relationship between mean water temperature and air temperature corresponds to an increase when compared with S9, both in the explanatory power (from 0.78 to 0.88) as well as in the slope (from 0.52 to 0.84), except for the site affected by the WWTP effluents (S5). Also, Lowney (2000) and Steel and Lange (2007) showed that daily water temperature fluctuations became narrower, and the time reaching maximum temperature delayed downstream of a dam when influenced by the release of hypolimnetic water with steady water temperature. Fluctuations of daily water temperature as well as of the time reaching minimum and maximum temperature were faster than in natural conditions because of reduced water volumes and differences in water abstraction with season.

Effects of the WWTP effluents on the downstream sites depend on the amount of wastewater released, on the stream discharge and on the seasonal rainfall. For example, water and air temperatures were not related downstream of a hydroelectric power plant in Minnesota (Erickson and Stefan, 2000). By contrast, Kinouchi et al. (2007) observed high correlations between water and air temperatures in spite of urban heat inputs from wastewater in a stream. In our study, even though the differences of air–water temperature relationship were observed downstream of WWTP effluents compared with other sampling sites, the explanatory power (0.84–0.87) as well as slope (0.73–0.79) of linear regressions were higher than in adjacent sites. This is likely due to the long time waste water spends in the shallow, unshaded treatment ponds, which favor the air-water heat exchange. Although the effects of effluents changed daily and seasonally, the site upstream of the plant (i.e. S4) usually formed isolated pools or completely dried up for several weeks in summer, whereas constant effluent outflows into S5 maintain a permanent flow even in summer (Ortiz and Puig, 2007).

The natural flow regime has been severely altered in many Mediterranean streams and rivers, and more than 80% of total water usage is for irrigation (Sabater, 2008; González del Tánago et al., 2012). Water abstraction for irrigation causes unexpected alterations of temperature fluctuations as well as of flow regimes and shifts permanent streams into temporary (or ephemeral) streams. These changes result in habitat simplification, deterioration of water quality and biodiversity losses. In addition, during dry months (summer), effluents from WWTPs maintain flow downstream of weirs. Our results suggest that the uncountable, minor hydrological alterations of Mediterranean streams substantially influence water temperature regimes. Further studies are needed to understand the effects of these thermal alterations on ecosystem function and structure.

CHAPTER 3

WATER ABSTRACTION AFFECTS ABUNDANCE, SIZE-STRUCTURE AND GROWTH OF TWO THREATENED CYPRINID FISHES

Merciai R., Molons-Sierra C., Sabater S., García-Berthou E. Water abstraction affects abundance, size-structure and growth of two threatened cyprinid fishes (*manucript under revision in the journal Plos One*).

Abstract

Hydro-morphological alteration is a major threat to freshwater biota, and particularly fish, in many river courses around the world. We analysed and compared the effects of water abstraction on two threatened cyprinid fishes of contrasting ecology (the Mediterranean barbel *Barbus meridionalis* and the Catalan chub *Squalius laietanus*) in a Mediterranean stream. We compared abundance, size-structure, growth, and condition of both species across perennial and artificially intermittent reaches affected by water abstraction. Both species were less abundant, had scarce large individuals, and displayed slower growth rates (length-at-age) in intermittent reaches, showing clear detrimental effects of water diversion. Mixed-effect models of scale increments showed different results than analyses of length-at-age. The larger-sized, water-column species (chub) disappeared or was rare in many intermittent reaches. The barbel present in intermittent reaches showed better somatic condition than in sites with permanent flow, perhaps due to reduced competition after rewetting or colonization by better fit individuals. This benthic, rheophilic species seems more resilient to moderate water abstraction than chub. Many effects of water flow intermittency were only detected on fish life-history traits when accounting for natural, often non-linear, variation, along upstream-downstream gradients. Our results suggest that abundance was the strongest indicator of effects of water abstraction on fish populations, whereas condition was a more labile trait, rapidly recovering from anthropogenic disturbance.

1. Background

Water scarcity is a main concern in intensively managed regions with low rainfall, where many small water courses often run partially or totally dry both because of lack of precipitations during extended periods of the year and water abstraction for human activities (Sabater and Tockner, 2010; García-Ruiz et al., 2011). In the particular case of Mediterranean-climate areas, drought events have increased in intensity and frequency during the last decades and are expected to persist as a consequence of climate and land use changes (Sabater and Tockner, 2010; Cooper et al., 2013; Dudgeon, 2013).

When dryness exceeds its natural variability due to human activities, physiological and behavioral adaptations of freshwater biota to overcome this kind of disturbance may no longer be effective to ensure the survival of populations (Gasith and Resh, 1999). Habitat patches like pools, which usually provide refuge to aquatic life during the dry season, reduce their volume and disconnect to each other for longer times. Physical, chemical and biotic conditions become harsher, and extinctions at local scales may be common (Lake, 2003; Magoulick and Kobza, 2003).

Fish are among the taxonomic groups most affected by hydrologic alteration (Poff and Zimmerman, 2010; Hermoso and Clavero, 2011). Mediterranean rivers support low fish richness, but most of their species are endemic and threatened according to IUCN criteria (Smith and Darwall, 2006). Modifications of Mediterranean fish assemblages (e.g. changes of relative abundance and decline of native and sensitive species) due to man-induced flow regime disruption have been well documented (e.g. Pires et al., 1999; Aparicio et al., 2000; Magalhães et al., 2007; Benejam et al., 2010, 2014). Population and individual metrics may represent useful indicators of environmental perturbation in ecosystems with low species richness, such as Mediterranean streams (Vila-Gispert and Moreno-Amich, 2001; Benejam et al., 2010; Mas-Martí et al., 2010; Alexandre et al., 2014a, b). Less studies have been conducted about the consequences of flow reduction and intermittency on fish at the individual level, for instance on growth or condition (Alexandre et al., 2014b), mostly focusing on salmonids (e.g. Harvey et al., 2006, 2014; Davidson et al., 2010; Teichert et al., 2010).

Habitat quality and food availability mediate fish health, growth and somatic condition (e.g. weight-length relationship). Fish body condition is expected to be related with individual growth (Marshall and Frank, 1999; Spranza and Stanley, 2000) and population abundance (e.g. Patterson, 1992; Winters and Wheeler, 1994; Guy and Willis, 1995). Growth and condition are known to be affected by physiological stress (Pankhurst and Van Der Kraak, 1997) and disturbances like drought and its associated environmental fluctuations, like water temperature, food supply, oxygen concentration, and also riparian cover (e.g. Vila-Gispert and Moreno-Amich, 2000, 2001; Harvey et al., 2006, 2014; Mas-Martí et al. 2010; Alexandre et al., 2014a, b).

The objective of our study is to examine the consequences of water abstraction on two threatened cyprinid species with different ecological niche in a small Iberian stream, the Tordera (NE Spain). Previous studies conducted in the same water course showed marked consequences of water abstraction on hydrology, thermal regime and fish assemblage (Benejam et al., 2010; Bae et al., 2016). Here we analyse the effects on fish at population and individual levels, comparing abundance, size, growth and body condition between intermittent and perennial reaches. Moreover, the respective contributions of fish age, sampling site and year to fish annual growth were assessed through the use of mixed-effects linear models (Weisberg et al., 2010). Fish metrics at individual and population level may represent a direct measure of health of fish and of the ecosystem they live in. Their alteration may reveal the effects of anthropogenic disturbance and may be especially useful in ecosystems with low species richness, like Mediterranean streams, in which the effectiveness of metrics at a community level may be low due to the scarce number of native species (Bramblett & Fausch, 1991; Ferreira et al., 2007). We expected to find lower fish densities due to summer mortality in the sites impacted by severe flow intermittency (e.g. Magalhães et al., 2007; Benejam et al., 2010, 2014), as well as lower growth rates and body condition due to physiological stress and increased competition for food during oversummering, because of harsh physico-chemical conditions and overcrowding in refugia (e.g. Oliva-Paterna et al., 2003a, b; Harvey et al., 2006, 2014; Mas-Martí et al., 2010). Finally, lower sizes were expected in intermittent sites, as a consequence of higher seasonal overall mortality (Magoulick and Kobza, 2003) and size-selective mortality due to physiological stress (e.g. Magalhães, 1993; Fernández-Delgado and Herrera, 1995).

2. Materials and Methods

2.1 Study area

Fish were sampled at fifteen sites in the Tordera Basin (see Methodology section for more details), of which twelve on the Tordera mainstem (T1 – T12) and three on the Arbúcies tributary (A1 – A3) (Fig 1, Table 1). Sites T1 to T3 had a scarcely anthropized habitat with fast-flowing water, stony substrate and dense canopy cover. Site T3 was located immediately upstream of a weir, where a channel diverts about 90 L s^{-1} for irrigation and urban use. In summer and early fall, most of the stream water is diverted into the channel and the downstream reach mostly dries or shows no discharge (Benejam et al., 2010; Bae et al., 2016). Consequently, sites T4 to T7 were affected by seasonal dryness aggravated by water diversion, with a few, small isolated pools usually persisting in this reach during the driest months, fed by groundwater. These pools offer refuge to fish, that reach high densities until water flow resumes. From T8 to T11 the stream flows across several urbanized and industrial areas. In particular, most summer water flow in T8 was constituted by the effluent of an urban wastewater treatment plant (WWTP) situated one km upstream, whereas T9 was located in the city of Sant Celoni. Site T12 was located in a reach also strongly impacted by diffuse water abstraction. These variations in water flow also affect the thermal regime along the Tordera course, especially in the intermittent reaches (Bae et al., 2016). In these hydrologically-altered reaches, daily temperature variation is significantly higher and the relationship between air and water temperatures is weaker, due to the reduction of the water volume coming from upstream, with consequent higher influence of ground water at more constant temperature. Moreover, in T8 the WWTP input disrupts the natural daily temperature pattern (Bae et al., 2016). Being located in a mountainous area, the Arbúcies tributary is much less affected by anthropogenic pressure, in comparison with the main basin of the Tordera. Complete dryness was never observed in sites A1 to A3 from 2004 to present.

Table 1. Physical and chemical features of the study sites at the Tordera stream and one of its tributaries (Arbúcies stream). Average values of water properties during the study period (May 2012 – May 2013) are shown. River width and maximum depth were measured every 10 meters along 100-m transects.

	Site	UTM (31T)	Altitude (m a.s.l.)	Mean width (min-max) (m)	Mean maximum depth (min-max) (cm)	Conductivity (μs cm^{-1})	pH	O_2 (mg L^{-1})	O2 (%)	Temperature (min - max) (°C)	Discharge (L s^{-1})	Flow regime
Tordera	T1	450056, 4622372	340	6.35 (5.24 - 6.97)	56.60 (49.1 - 65.9)	105.24	7.87	9.64	94.73	-	149.3	Permanent
	T2	450439, 4621726	319	6.82 (6.17 - 7.24)	60.81 (54.8 - 73.2)	110.03	7.88	9.77	95.27	-	309.5	Permanent
	T3	450862, 4620754	305	5.87 (4.95 - 6.89)	40.52 (31.9 - 53.2)	109.27	7.64	9.64	93.60	12.33 (4.39 - 19.56)	291.9	Permanent
	T4	451946, 4619585	267	4.98 (2.70 - 5.83)	29.98 (26.0 - 35.6)	119.80	7.89	9.46	90.22	9.83 (4.07 - 18.91)	167.7	Temporary
	T5	451666, 4619724	262	5.32 (3.84 - 7.36)	35.99 (25.2 - 44.6)	116.69	7.82	10.04	96.57	-	106.0	Temporary
	T6	452988, 4617886	213	6.53 (5.20 - 8.56)	29.80 (22.3 - 36.6)	113.24	7.42	9.79	95.38	-	152.5	Temporary
	T7	454803, 4615455	173	6.06 (5.70 - 7.09)	26.42 (21.9 - 29.9)	135.24	7.67	9.81	97.02	11.13 (7.14 - 19.06)	217.0	Temporary
	T8	455681, 4614381	135	7.28 (5.05 - 11.94)	41.67 (31.5 - 54.9)	361.66	7.64	8.05	84.39	15.56 (7.25 - 24.19)	212.7	Permanent
	T9	459053, 4615859	120	7.32 (6.02 - 9.87)	50.73 (39.6 - 61.8)	457.71	7.74	8.49	86.90	15.11 (8.51 - 20.59)	180.3	Permanent
	T10	467054, 4619832	67	9.34 (3.89 - 13.9)	45.11 (19.1 - 68.7)	461.00	7.63	7.98	82.50	19.16 (12.85 - 22.61)	-	Permanent
	T11	474643, 4620999	36	16.61 (7.25 - 26.41)	39.85 (21.8 - 62.2)	514.50	8.13	9.24	97.35	20.60 (12.93 - 24.13)	-	Permanent
	T12	481311, 4611807	6	32.17 (15.3 - 49.05)	43.45 (37.0 - 49.9)	510.00	8.16	9.38	99.85	14.87 (6.46 - 25.13)	-	Temporary
Arbúcies	A1	456886, 4630614	386	3.93 (3.82 – 4.44)	33.23 (28.80 - 40.5)	245.57	8.21	9.55	94.37	-	-	Permanent
	A2	462250, 4627821	212	6.32 (4.21 - 7.10)	32.51 (25.3 - 43.8)	296.27	8.03	9.54	93.87	13.80 (5.02 - 22.3)	458268.8	Permanent
	A3	467259, 4623025	91	4.96 (2.54 - 6.75)	25.17 (11.6 - 40.9)	310.33	8.24	9.84	94.37	-	-	Permanent

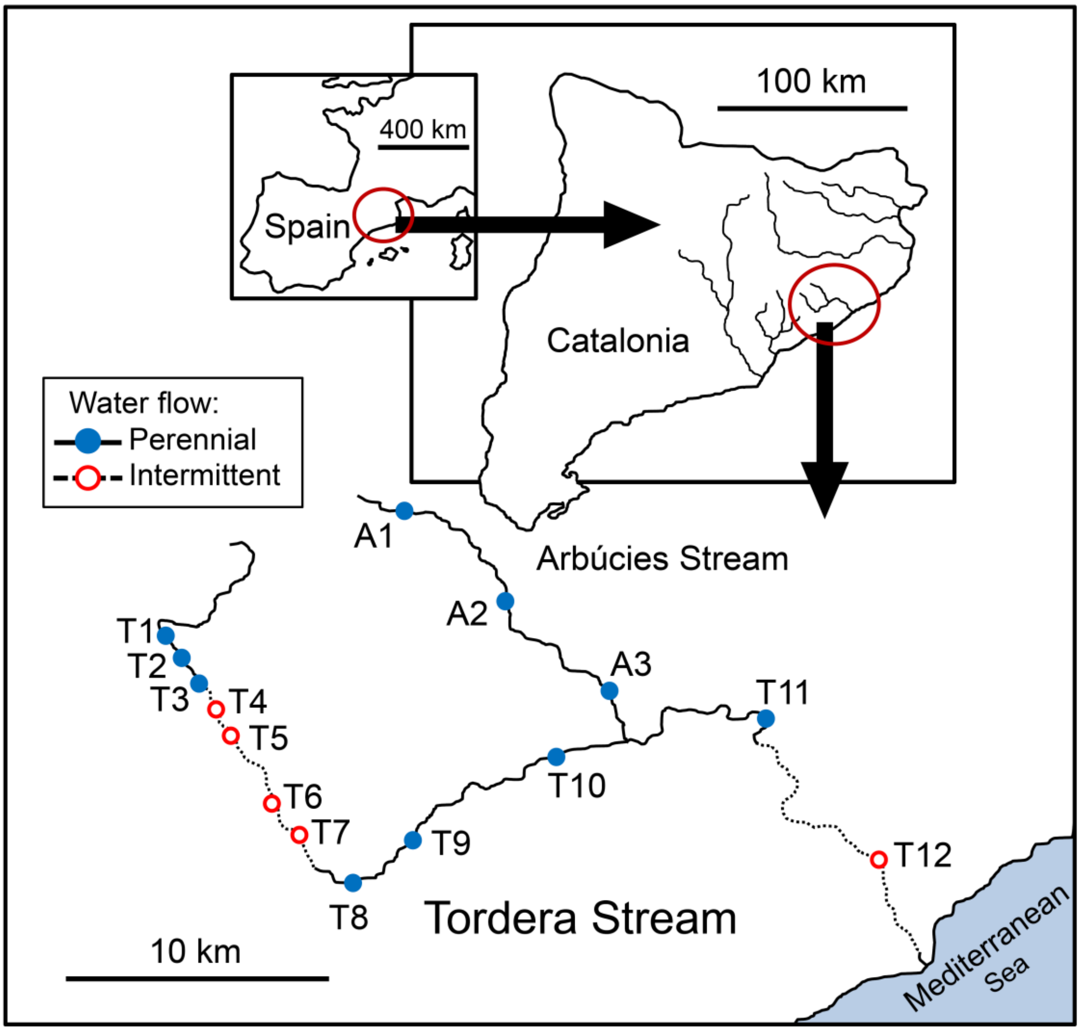


Fig. 1. Location and map of the study area: Tordera (T1-T12) and Arbúcies (A1-A3) streams. The flow regime during the study period is also shown. See Table 1 for further details on the sampling sites.

2.2 Fish sampling and age determination

Fishing permits were granted by the Catalan Government to RM and EGB for fish sampling. The permits allowed the capture for scientific purpose of live fish of any species with no quantity restrictions, including threatened species, on the Catalan territory excluding protected areas. Electrofishing, netting and trapping were permitted. EGB has proved to the satisfaction of the Catalan Autonomous Ministry of Agriculture, Livestock, Fisheries, Food and Natural Environment that he meets the requirements necessary to carry out the tasks of scientist (person responsible for directing animal experiments) in accordance with the Catalan Government Decree 214/1997 of July 30th, regulating the use of animals for experimental and other scientific purposes. No fish were sacrificed for this study. All fish were captured by electrofishing, anesthetized to avoid unnecessary handling stress, then released in the capture site after recovery in freshwater tanks. Therefore, no further permission is necessary from Ethics committees and research was conducted according to relevant national and international guidelines.

In each study site, a 100-m reach was sampled by one-pass electrofishing (Smith-Rooth LR-24 backpack electrofisher, 100-150 V, 0,8-1,5 A, fully rectified triphasic DC) every two months from May 2012 to October 2013. Fish abundance was estimated as catch per unit effort (CPUE). A comparison of our sampling protocol with four-pass removal with block nets in streams of this region indicated that: (i) 50–100% of the species and 40–60% of the individuals are generally captured with a single pass; and (ii) that the estimates of species richness and composition of a single pass are represenative of the assemblage (Benejam et al., 2012). A total of 1754 *Barbus meridionalis* and 765 *Squalius laietanus* were captured, anesthetized with 5-10 drops of clove oil (Aura Cacia® pure essential oil) diluted in 10 L of water, measured to the nearest mm (fork length) and weighed to the nearest 0.1 g. Scale samples (5-10 each) of 860 barbel and 406 chub were collected from the same area above the lateral line and posterior to the dorsal fin. All fish were released in the capture site after recovery from electrofishing.

In the laboratory, fish scales were immersed for 30-60 minutes in a 5% KOH aqueous solution to eliminate skin remains, then mounted on glass slides and observed with a microfiche reader for age determination, discarding regenerated scales (García-Berthou and Moreno-Amich, 1992). An annulus was taken as a transition between two uninterrupted zones of closely- and widely-spaced circuli. In the case of chub an essential criterion for annulus identification was anastomosis, namely several circuli which cut across (cutting-over) several others on the rest of the scale (Chugunova, 1963). To estimate ageing precision, a subsample of 50 scales for each species was examined independently by two researchers. Radius length was measured on the oral side of the scale for every annulus recognized, to determine the annual fish scale size increment, i.e. the distance between two annuli on the fish scale (Francis, 1990).

2.3 Statistical analyses

The main statistical analyses to test for the effects of flow regime on all response variables consisted in building minimum adequate models (MAM) with linear models

(Crawley, 2002). We simplified MAMs for the response variables (site averages for density, lengths, condition, growth (size-at-age), and scale increment) using altitude as a covariate, and flow regime (perennial vs. intermittent) as a categorical factor. These initial analyses of covariance (ANCOVAs) also included a quadratic component of altitude, to account for nonlinear natural variation along the stream (see e.g. Carmona-Catot et al. 2010, 2011 for a similar approach), and interactions between covariates and flow regime. We simplified terms in the models by removing all non-significant interactions and main effects ($P > 0.1$).

To analyse fish condition (weight-length relationship), we used ANCOVAs of weight as the response variable, sampling site and season as categorical factors, and FL as the covariate. FL and weight were $\log_{10}$-transformed, to satisfy the statistical assumptions (normality, homoscedasticity, and linearity). ANCOVAs were also used to compare fish growth among different sampling sites and seasons (categorical factors), with fork length (FL) as dependent variable and age as covariate. All ANCOVA models included interactions among covariates and categorical factors, which test the equal slopes assumption of standard ANCOVA, and were only removed if they were non-significant ($P > 0.1$), following García-Berthou and Moreno-Amich (1993). Estimated marginal means of the dependent variables are the means for each level of the factor, adjusted for covariates with ANCOVA, and were used to describe the differences in fish condition and growth across sites (García-Berthou and Moreno-Amich, 1993). All ANCOVAs were performed with SPSS 20.

Independent age estimations underwent Bowker’s test for symmetry (Hoenig et al., 1995) and *t*-tests to check for systematic bias between the readers’ interpretations, using the functions “ageBias” and “agePrecision”, respectively, of the “FSA” package (Ogle, 2013) of the R environment (R Core Team, 2011). We also computed the coefficient of variation (CV) of age readings as a measure of precision by using the same package.

The effects of age, site and year on fish yearly size increment were estimated with mixed-effects linear models (MELMs), following Weisberg and co-authors (Weisberg et al., 2010; Weisberg, 2012). This method has fewer assumptions than others based on fish length back-calculation, analysing the annual fish scale size increment (dependent variable) with no need of a particular relationship between scale radius and fish size. Age was considered as a fixed-effect factor, whereas site, year of growth, fish individual and their interactions were treated as random-effects factors. Analyses were performed using the function “lmer” of “lme4” R package (Bates et al., 2013) and the function "rand" of "lmerTest" package (Kuznetsova et al., 2013). Moreover, we searched for relationships between the contribution of the year of growth to scale size annual increment and climatic features (yearly averages of air temperature, precipitation, stream flow, proportion of days of drought per year) measured at various sites in the watershed (Data from ACA: https://aca-web.gencat.cat/aca/appmanager/aca/aca/) starting from 2004, year in which the oldest fish found were born.

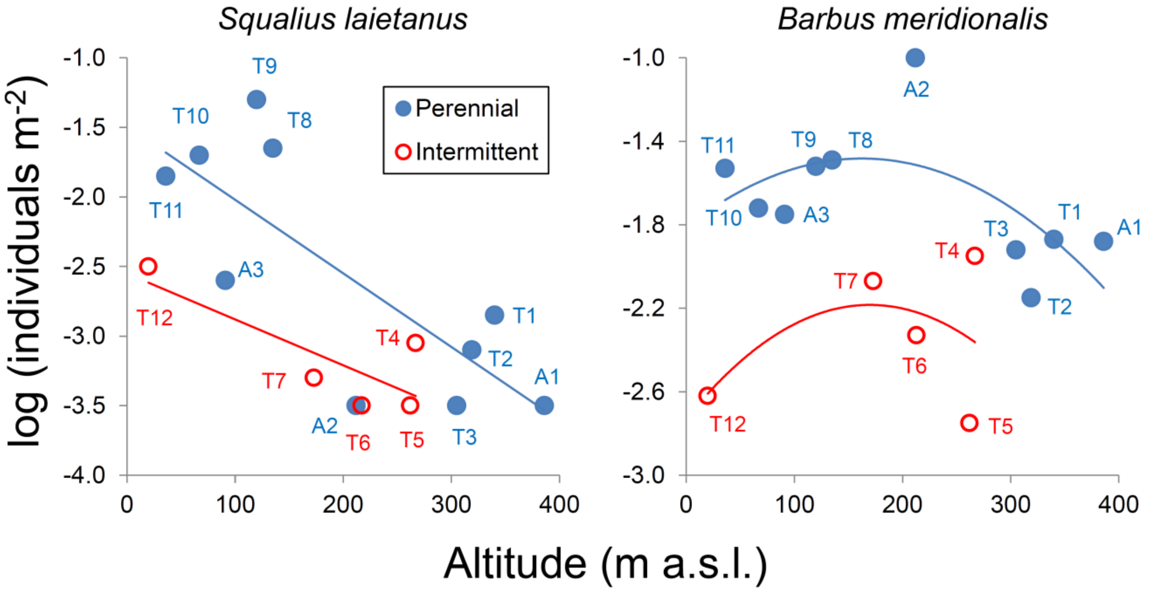


Fig. 2. Relationship of mean fish abundance (May 2012 - October 2013) with altitude by flow regime and fish species. The lines correspond to linear (*S. laietanus*, top plot) or quadratic regressions (*B. meridionalis*, bottom plot) separated by flow regime. Linear r^2 are 0.668 (perennial) and 0.620 (intermittent). Quadratic R^2_{adj} are 0.407 (perennial) and 0.223 (intermittent). Sites T1-T12 are located on Tordera mainstem; sites A1-A3 are on the Arbúcies tributary. *Squalius laietanus* was not captured at sites T3, T5, T6, A1, and A2.

3. Results

Many response variables displayed nonlinear variation along the upstream-downstream gradient, and this was considered in order to properly test the effects of flow regime (Table 2). Barbel density showed a quadratic trend, peaking in the middle course of both Tordera and Arbúcies streams (Fig 2). By contrast, chub density fitted a linear model showing higher values in the middle and low course of Tordera mainstem, with scarce appearance in other sites (Fig 2). Chub specimens were not found at sites T3, T5, T6, A1 and A2. The two species were less abundant in the intermittent reaches. Barbel densities (mean CPUE ± SE) were 53.9 ± 17.3 in intermittent and 280 ± 88.7 fish ha^{-1} in perennial reaches. Chub densities were 7.24 ± 5.5 in the intermittent and 111 ± 53.5 fish ha^{-1} in the perennial reaches. The effect of water intermittency was highly significant for the two species after accounting for the variation with altitude, with around 60% of explained variation (R^2_{adj}) for both models (Table 2).

Table 2. Minimum Adequate Models (MAMs) of six population and individual features (response variables; see text for details) of *S. laietanus* and *B. meridionalis* in the Tordera basin in 2012 and 2013. The linear models tested the effect of Flow Regime (FR) (categorical factor) controlling for altitude (Alt.) (linear and quadratic effects). Terms that showed no significant effects ($P > 0.1$) were removed from the model. The P value corresponds to the effects in the selected model of the flow regime (perennial vs. intermittent).

Response variable	MAM	P (FR)	R^2_{adj}
S. laietanus			
Density	FR + Alt.	0.001	0.600
Mean FL	FR × Alt. × $Alt.^2$	0.065	0.916
Maximum FL	-	-	-
Condition	-	-	-
Growth	-	-	-
Scale Increment	-	-	-
B. meridionalis			
Density	FR + Alt. + $Alt.^2$	0.001	0.604
Mean FL	-	-	-
Maximum FL	FR × Alt. × $Alt.^2$	0.005	0.844
Condition	-	-	-
Growth	FR × Alt. × $Alt.^2$	0.014	0.416
Scale Increment	FR × Alt. × $Alt.^2$	0.014	0.601

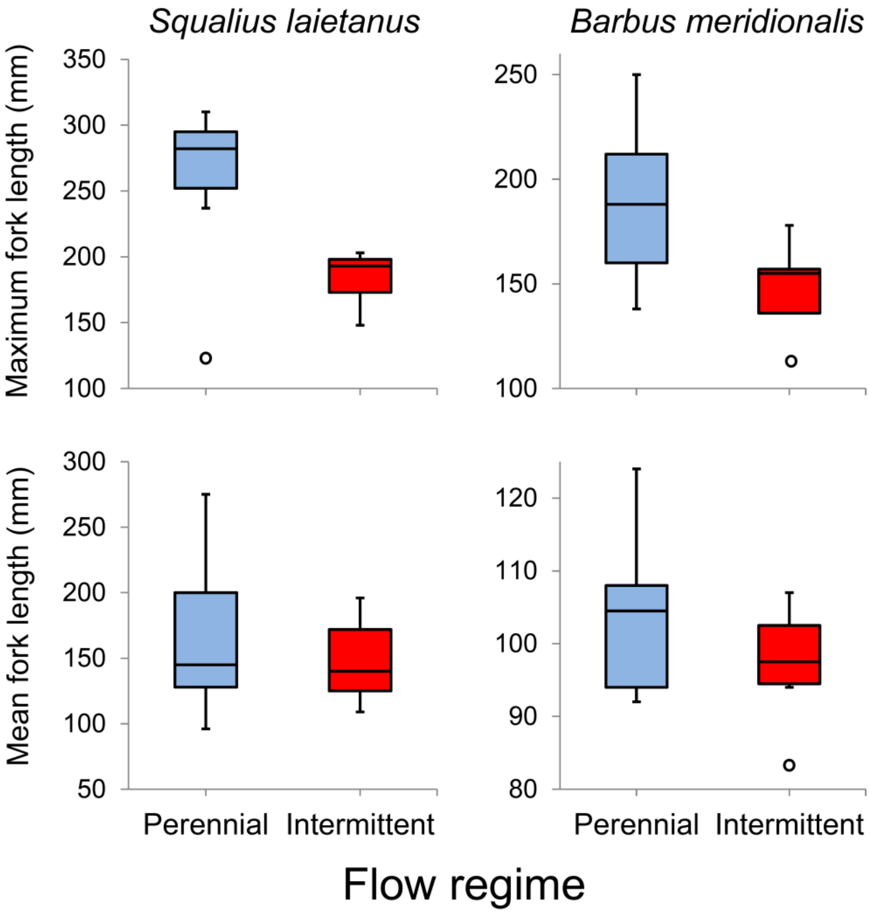


Fig. 3. Maximum and mean size of fish captured in the Tordera basin during 2012 and 2013, comparing perennial and intermittent reaches. Boxes represent the first and third quartiles, lines are the medians, and bars are maximum and minimum values, excluding outliers (circles).

Quadratic models of altitude with interacting effects of flow regime were selected for mean length of chub and maximum length of barbel, both explaining high percentages of variation (> 84%) and with observed lengths lower for both species in the intermittent reaches (Fig 3). Both species displayed among-site variation in fish condition, with significant site × length interaction indicating different slopes of the weight-length relationship (Table 3). Barbel condition displayed a quadratic variation with altitude (Fig 5) similar to abundance and was marginally higher in the intermittent than in perennial reaches (P = 0.07, *t*-test) (Figg 4 and 5). Similarly to abundance, chub condition was highest in T8-T9 and progressively decreased along the stream, from the middle reaches to the lowermost and intermittent reach (Fig 6). Chub condition displayed significant among-site and seasonal variation and also site × length interaction. Although age estimation varied moderately among readers (CV of 9.7% for barbel and 7.9% for chub), there were no significant asymmetries in the age readings of barbel (Bowker's test: $\chi^2 = 8.8$; df = 4; $P = 0.066$) and chub age ($\chi^2 = 6.3$; df = 6; $P = 0.39$) and no systematic biases (*t*-tests, $P > 0.3$) (Fig 4). Both species also showed significant variation in growth (length-at-age) among sites and with site × length interaction (Table 3). Barbel growth (length-at-age) also showed quadratic variation with altitude, interacting with flow regime (Table 2), and slightly lower age-adjusted fork length in intermittent reaches. The intermediate reaches T8 and T9 showed high rates of barbel growth (Fig 7), in agreement with the results of density and condition. Chub growth was higher in permanent reaches that in the intermittent reach, where it was more abundant (Fig 7).

Table 3. Analyses of covariance of condition (top) and growth (bottom) of chub and barbel caught in the Tordera basin (May 2012- October 2013). The first analysis tested differences of total weight (dependent variable) across sites and seasons (categorical factors), and their interactions, controlling for fork length (covariate). The second analysis tested the effects of sampling season and site (categorical factors), and their interactions, on fork length (dependent variable) controlling for age (covariate). Total weight and fork length were log-transformed.

	S. laietanus				*B. meridionalis*			
	$R^2_{adj} = 0.988$				$R^2_{adj} = 0.978$			
Total weight	SS	df	*F*	*P*	SS	df	*F*	*P*
Fork length (FL)	22.95	1	6667.0	< 0.0005	15.94	1	5071.0	< 0.0005
Site	0.117	3	11.34	< 0.0005	0.096	14	2.173	0.007
Season	0.144	5	8.398	< 0.0005	0.016	2	2.510	0.082
Site × FL	0.124	3	12.00	< 0.0005	0.076	14	1.729	0.045
Season × FL	0.155	5	9.007	< 0.0005	0.010	2	1.610	0.200
Site × Season	0.180	9	5.807	< 0.0005	0.005	3	0.576	0.631
Site × Season × FL	0.181	9	5.853	< 0.0005	0.004	3	0.421	0.738
Error	1.552	451			2.556	813		
	$R^2_{adj} = 0.596$				$R^2_{adj} = 0.686$			
Fork length	SS	df	*F*	*P*	SS	df	*F*	*P*
Age	1.445	1	181.6	< 0.0005	0.734	1	156.9	< 0.0005
Season	0.012	2	0.742	0.477	0.031	2	3.284	0.038
Site	0.120	4	0.005	0.005	0.417	14	6.372	< 0.0005
Season × Site	0.006	2	0.352	0.703	0.083	3	5.929	0.001
Season × Age	0.017	2	0.017	0.336	0.015	2	1.591	0.204
Site × Age	0.125	4	3.943	0.004	0.351	14	5.364	< 0.0005
Season × Site × Age	0.014	2	0.904	0.406	0.156	3	11.15	< 0.0005
Error	2.760	347						

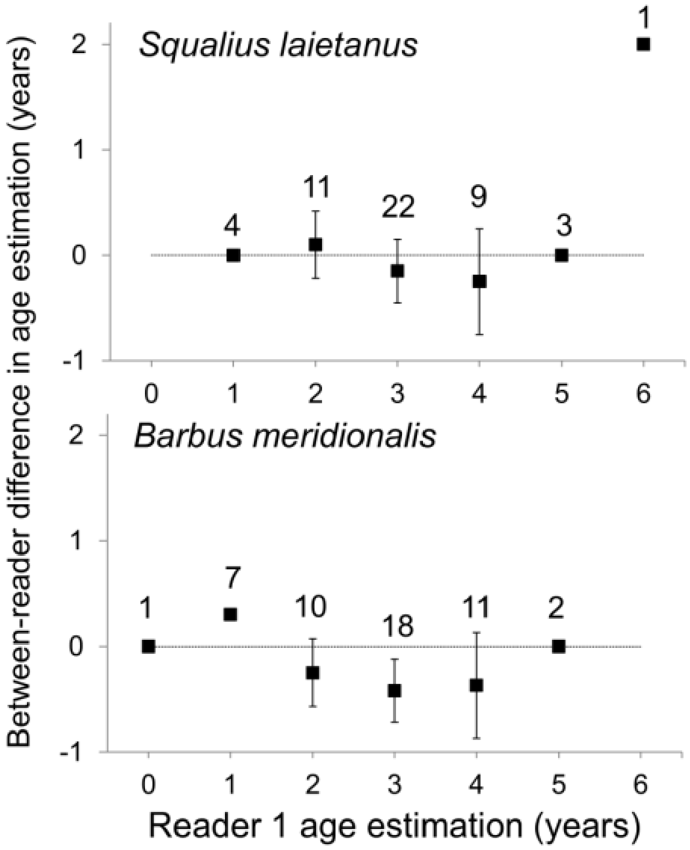


Fig. 4. Between-reader differences in age estimation (mean and SEs are shown). Numbers above error bars are the number of fish.

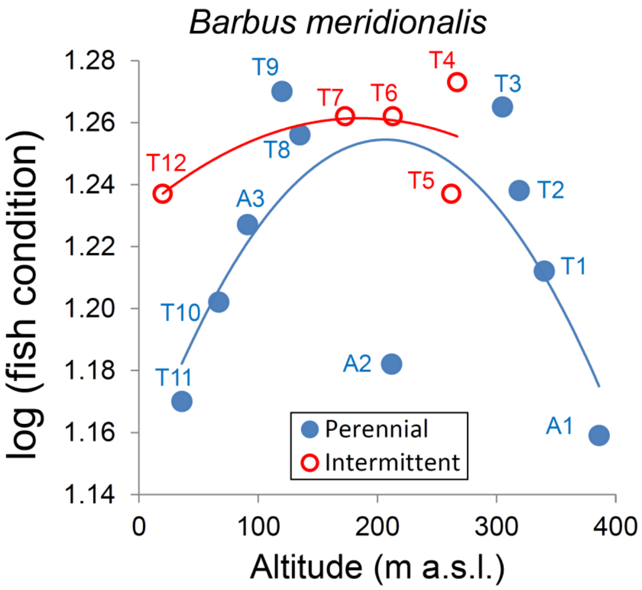


Fig. 5. Relationship of fish condition (total weight adjusted for fork length with analysis of covariance) of *B. meridionalis* with altitude. The lines correspond to quadratic regressions by water flow regime. Total weight and fork length were log-transformed. Quadratic R^2_{adj} was 0.406 for perennial reaches and 0.375 for the intermittent reaches.

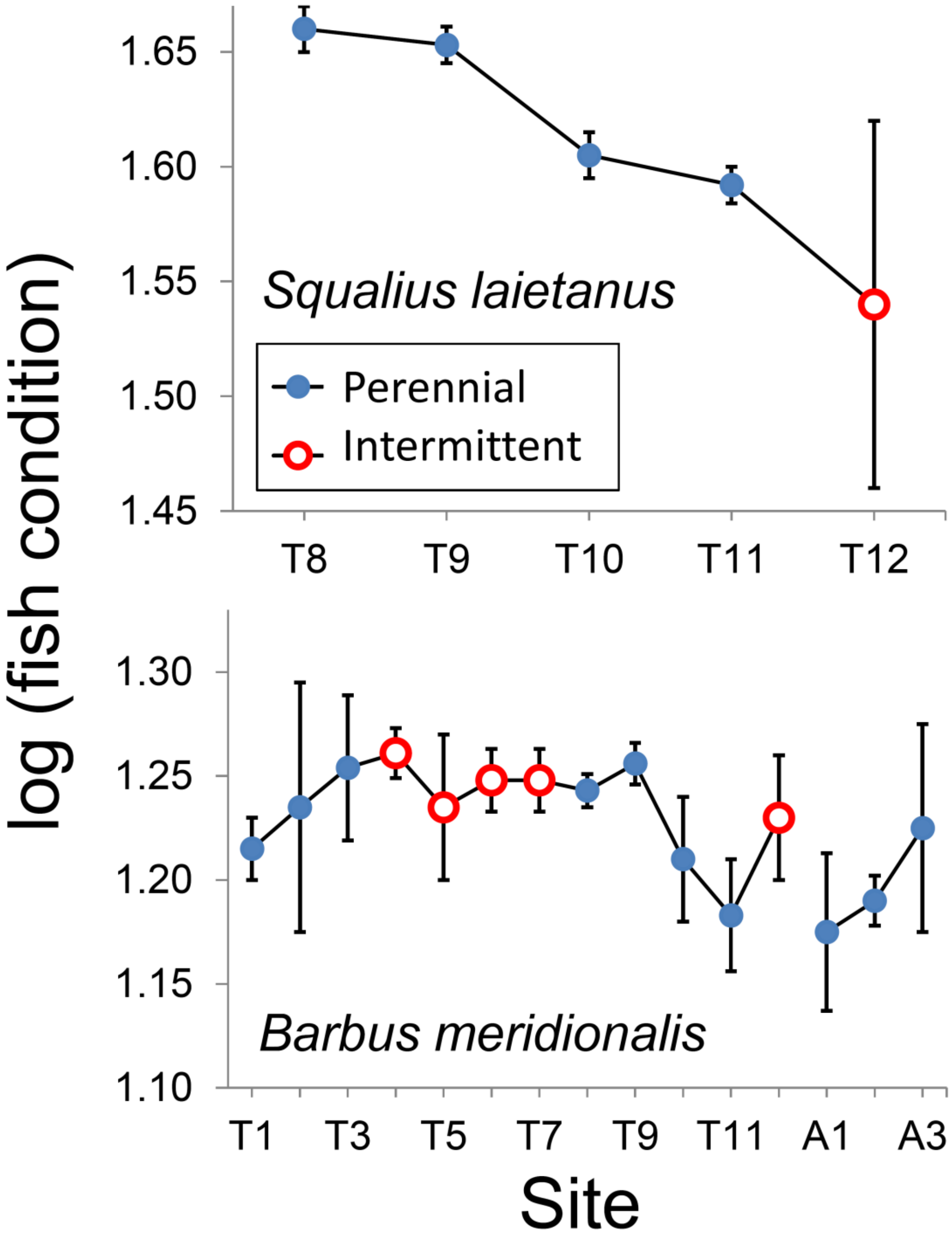


Fig. 6. Marginal means of fish condition (total weight adjusted for fork length with analysis of covariance) across sampling sites (Table 2). The mean $\log_{10}$ of fork length (at which fish total weight was adjusted) was 2.165 for chub and 2.01 for barbel. Non-estimable means are not shown. Sites T1-T11 are located on Tordera mainstem and sites A1-A3 are on the Arbúcies tributary.

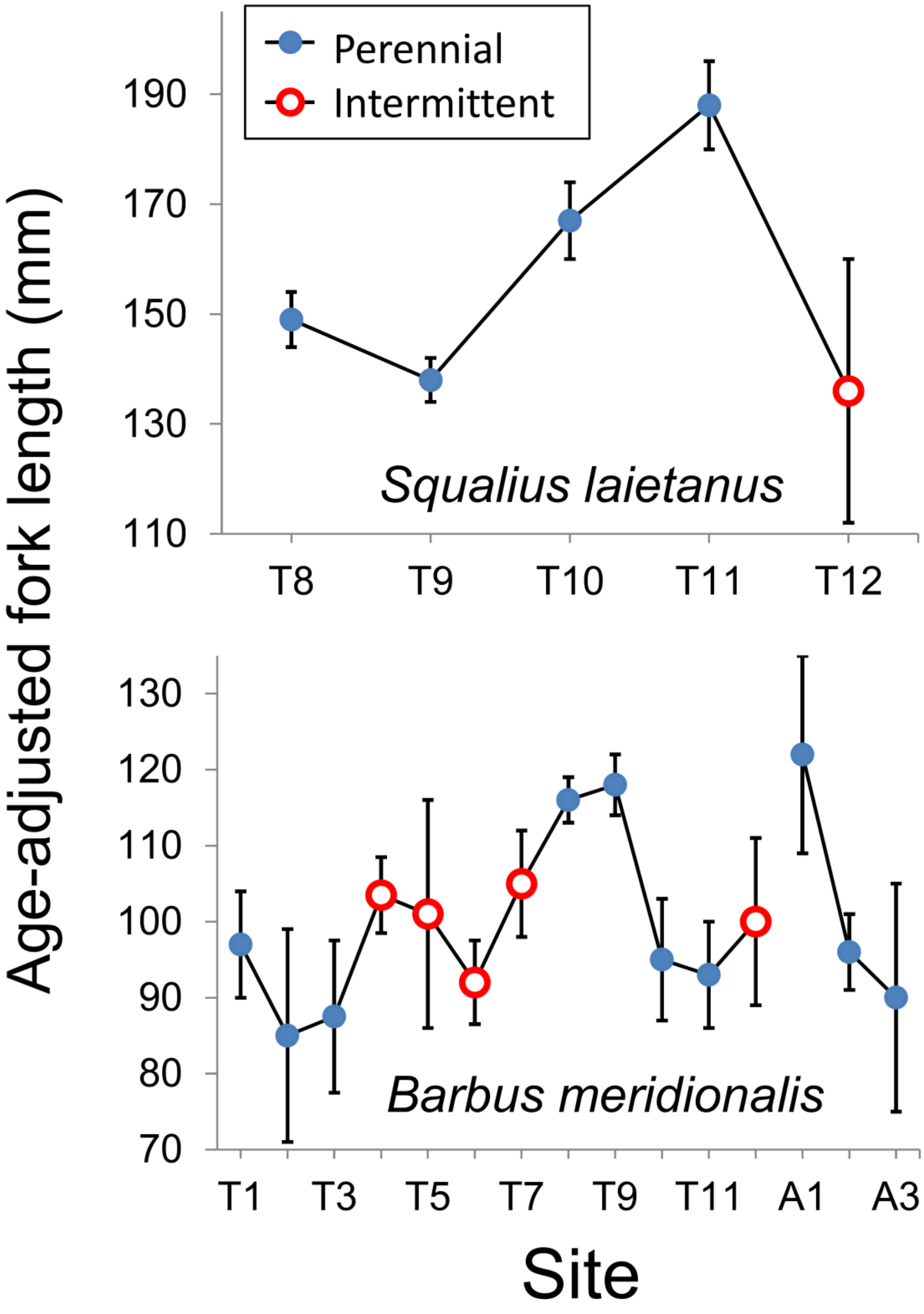


Fig. 7. Marginal means of fish fork length (adjusted for fish age with analysis of covariance) across sampling sites (Table 2, below). The mean age (at which fish size was adjusted) was 2.82 years for *S. laietanus* and 2.83 years for *B. meridionalis*. Non-estimable means are not shown. Sites T1-T11 are located on Tordera mainstem; sites A1-A3 are on the Arbúcies tributary.

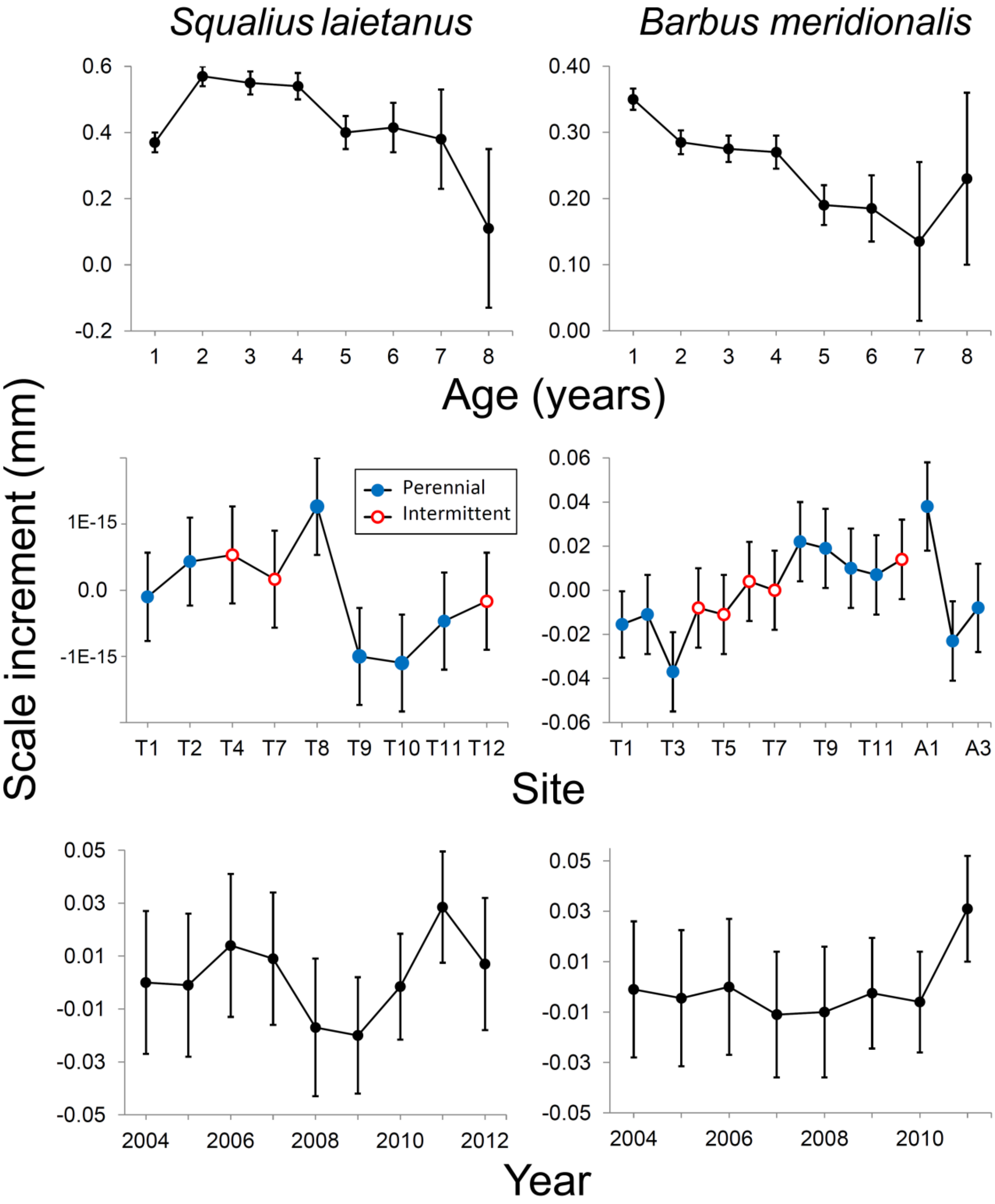


Fig. 8. Effects of fish age, sampling site and year of growth on yearly increments of fish scale oral radius, as estimated by the mixed-effects models. See Table 3 and text for significance of the factors. The bars how standard errors. Codes of sampling sites as in Table 1.

Barbel annual size increment was significantly affected by flow regime, as highlighted after accounting for the effect of squared altitude (Table 2). The average increment of scales for barbel showed significant age × site × year, age and individual effects (Table 4) and was slightly higher in reaches with perennial water flow (0.0004 *vs*. -0.0008 mm). Scale increment showed a general upstream-downstream increase upstream-downstream trend in the Tordera mainstem (Fig 8). In the case of chub, age × site × year and individuals effects were significant (Table 4) and scale increment was slightly higher in intermittent reaches.

For the two species, age × site × year and individual were the most important sources of variation (Table 4). The youngest age classes (1 and 2 year old) had the highest average growth rates, which declined with age. Environmental factors seemed favourable to barbel growth in 2012, whereas the best growth year for chub was 2011, after three years of slowest growth (Fig 8). Barbel annual size increment was positively correlated to the proportion of days with discharge equal to zero recorded in T9 (Pearson's r = 0.773; P = 0.024), whereas a marginally significant correlation (r = 0.602; P = 0.082) was found between chub increment and air temperature measured in St. Celoni, close to T9.

Table 4. Mixed-effects linear models of size increments in fish scales collected between May 2012 and October 2013. Fish age was treated as a fixed-effect factor, whereas year of growth, sampling site, fish individual, and interactions were treated as random effects.

	Squalius laietanus			*Barbus meridionalis*		
Random effects	χ^2	df	P	χ^2	df	P
Year	1.830	1	0.180	1.763	1	0.184
Site	0.000	1	1.000	4.406	1	0.036
Year × site	0.000	1	1.000	2.836	1	0.092
Age × site	0.000	1	1.000	0.849	1	0.357
Year × age	0.000	1	1.000	2.588	1	0.108
Age × site × year	5.770	1	0.020	10.02	1	0.002
Fish individual	3.920	1	0.050	17.74	1	< 0.0005

4. **Discussion**

4.1 Effects of water abstraction

The density of the two species was noticeably higher in the perennial reaches. Chub was the species most affected by water abstraction, and this caused its disappearance from a long intermittent reach where it was present historically (Benejam et al., 2008; 2010). However, more significant effects of water abstraction for other variables were obtained for barbel because it is more widespread in the basin, whereas chub is only present in some stream segments. Several cases of flow reduction have been associated to decreased fish abundance (e.g. Kubečka et al. 1997; Almodóvar & Nicola 1999; Benejam et al., 2014), as well as recent fish population contractions or local extinctions related to hydrologic alteration in the Tordera catchment (Aparicio et al., 2001; Aparicio and Vargas, 2004; Benejam et al., 2010; Otero et al., 2011). Considerable numbers of dead fish of several species (including the two study species as well as brown trout and *Phoxinus* sp.) were directly observed during the study period, mostly in reaches immediately after pool drying. On the other hand, high densities of barbel, with occasional presence of chub, were detected in pools persisting during summer, suggesting the existence of a drought-escape behaviour for these fishes. This behaviour has been described in regions affected by dry summers, where the initial phase of drying may promote fish movement (Matthews and Marsh-Matthews, 2003; Albanese et al., 2004). These observations allowed us to recognize streambed dryness as a leading cause of low fish density in these reaches, associated to death and migration (Davey and Kelly 2007; Magalhães et al. 2007).

Natural variation in fish longitudinal distribution can in part explain the observed pattern of fish survival, since lower reaches have more stable ecological conditions, are nutrient-rich and show higher water temperatures than the uppermost ones (Grossman et al., 1998; Matthews, 1998). In particular, the middle reaches showed the highest fish densities, probably because of both perennial flow regime but also because the higher nutrient input increases ecosystem productivity. Chub, moreover, is known to colonize preferentially deep pools and runs (Doadrio et al., 2011), and riffles are the most common habitat in the upper course of Tordera and Arbúcies, where few individuals of this species were found.

4.2 Effects of water intermittency at individual level

Our observations confirmed the presence of smaller individuals of barbel and chub in the Tordera intermittent reaches (Benejam et al. 2010). This pattern may be due to three possible factors: i) high mortality rates in intermittent sites, that imply lower probabilities for fish to live many years and attain large sizes; ii) higher mobility of larger individuals, potentially more capable to move away from impacted reaches (Magalhães, 1993; Albanese et al., 2004); and iii) size-selective mortality due to physiological stress, which is known from *Barbus*, *Squalius* and other Mediterranean cyprinids as a consequence of flow intermittency (Herrera and Fernández-Delgado, 1992, 1994; Magalhães, 1993; Fernández-Delgado and Herrera, 1995) because of higher oxygen demand of larger individuals (Klinger et al., 1982; Castleberry and Cech, 1986).

Fish condition and growth were expected to vary across sites and seasons, and also to be lower in the intermittent reaches. Such a response to low flow conditions has been reported for barbel *Luciobarbus* sp. (e.g. Oliva-Paterna et al. 2003a, b; Mas-Martí et al., 2010), salmonids (e.g. Deegan et al., 1999; Harvey et al., 2006), and smallmouth bass (Paragamian and Wiley, 1987). Our observations partially confirmed our hypothesis. Growth, measured as length-at-age, was lower at intermittent reaches for both chub and barbel. Scale increments did not confirm these results, possibly because increments in calcified structures are not always well correlated with growth rates (Secor & Dean 1989) and because the models used consider average scale increment in many growing seasons (accounting for many other factors) and not final size-at-age. Surprisingly, barbel from intermittent reaches showed better condition (weight-length relationship), comparable with the ones from the urbanized reaches in the middle Tordera course, in which fish of the two species looked healthy and abundant. Another study (Spranza and Stanley, 2000) reports both higher condition and growth of cyprinids in sites affected by summer dryness. Barbel populations from permanent streams, moreover, have been reported to show a slender body profile and lower condition than populations from intermittent streams, a likely adaptation to higher flow and water velocity (Alexandre et al., 2014). Fish inhabiting instable environments like intermittent streams may require high levels of energy reserves, i.e. high body condition, a likely investment to increase reproductive success (Moyle and Cech, 1996; Alexandre et al., 2014a, b). These independent evidences cannot exclude that high barbel condition in the Tordera intermittent reaches may also depend on adaptive phenotypic plasticity. In several other cases, by contrast, *Barbus* condition was reported to be positively correlated to riparian vegetation cover, water flow and related environmental variables, such as conductivity and oxygen concentration (e.g. Vila-Gispert et al., 2000; Vila-Gispert and Moreno-Amich, 2001; Oliva-Paterna et al., 2003a, b; Mas-Martí et al., 2010). Mas-Martí et al. (2010) attributed the lower condition of *B. meridionalis* and *S. laietanus* in an intermittent tributary of the Tordera to two main factors, namely the lower temperature and productivity of the tributary, and bottom-up effects of stream dryness on the trophic web, leading to lower food availability for fish. In our case, part of the upper intermittent reach was covered by a dense tree canopy, which coupled with hyporheic flow kept temperature and dissolved oxygen within tolerable levels, mitigating the physiological stress for fish. Moreover, strong competition for food was limited to some weeks in the summer refugia, but high invertebrate availability per capita could be guaranteed in the other seasons for the few fish that survived drying. Alternatively, fish with better condition might be more capable of recolonizing intermittent reaches. Overall, dryness increases mortality and decreases abundance and growth but fish present may show better individual condition due to better colonization capability or reduced resource competition.

Chub was usually present, at low densities, in only one of the intermittent sampling reaches, the lowermost one, making difficult to compare traits among impacted and control sites. Our results show a gradual condition decrease from the most urbanized sites to the lowermost, intermittent one, rather than a simple distinction between sites with different flow regimes. In fact, most life-history traits showed variation, often non-linear, with altitude which was essential to be accounted for the effects of flow regime. Density, growth, and condition of barbel were highest at intermediate altitudes. These patterns have been observed in many freshwater fishes but might also be due in part to anthopogenic factors. The middle reaches of the Tordera mainstem are a nutrient-rich zone affected by the efluents of WWTPs (Merseburger et al., 2005; Ortiz et al., 2006). These findings contrast with those of Britton et al. (2012), who found higher *Barbus* growth rates in presence of low phosphate loads, but match observations with other European cyprinids such as *Rutilus rutilus* (e.g. Lappalainen et al., 2001; Beardsley and Britton, 2012). Arguably, a moderate nutrient enrichment in the middle Tordera course may increase barbel growth and condition if they do not exceed the tolerance limits of this species. Chub increment also peaked in site T8, but the absolute contribution of site to the overall variability, according to the MELM, was negligible.

The growth-at-age of the two species may reflect sexual maturation. This occurs at age 1 for most individuals of barbel, whereas in chub it is more variable (Casals-Martí, 2005) and may correspond to the first growth decrease observed after the second year of life (Lobon-Cerviá, 1982; Geraldes and Collares-Pereira, 1995). Furthermore, as chub is a multiple spawner with reproductive season potentially protracted (Casals-Martí, 2005) we cannot exclude that a significant fraction of the new-borns hatch at the beginning of an unfavourable period, resulting in low growth rates in the first year (Geraldes & Collares-Pereira, 1995; Nunn et al., 2002, 2007; Bolland et al., 2007). Chub size increment was higher in warmer years: this was expected, since temperature is a dominant factor, together with food availability, in determining fish growth patterns in space and time (e.g. Lappalainen et al., 2001; Beardsley and Britton, 2012).

Our research confirmed the existence of strong effects of hydrologic alteration at the population and individual levels for the two study species. Population and individual metrics showed highest values in the middle Tordera course, where flow is permanent and nutrient availability is higher due to WWTP inputs. Body condition was not negatively affected by stream drying, revealing how individual traits vary in the potential to inform about hydrological regime disruption. Finally, it is important to stress how the effects of drying were often highlighted only after controlling for natural variability through appropriate statistical tools. *Squalius laietanus* was the species most affected by drought, being absent from most impacted sites. *Barbus meridionalis* seemed better adapted to take advantage of moderate environmental disturbance, probably through increased resistance and colonization ability, except in extreme situations in which it is wiped away. We would expect this species to recover quickly in terms of abundance in the intermittent reaches if drought events were less intense and lasting, whereas chub was revealed to be more sensitive to reduced stream flow and may be less resilient to hydrologic alteration.

CHAPTER 4

WATER DIVERSION REDUCES ABUNDANCE AND SURVIVAL OF TWO MEDITERRANEAN CYPRINIDS

Merciai R., Bailey L., Bestgen K., Fausch K., Zamora L., Sabater S., García-Berthou E. Water diversion reduces abundance and survival of two Mediterranean cyprinids (*article in preparation*).

Abstract

Water flow is a fundamental characteristic required for the ecological integrity of stream ecosystems. However, populations of many freshwater fishes in the Mediterranean area are threatened by man-induced drought due to water diversion. Mark-recapture methodology is an effective tool for estimating fish abundance and survival probability, but it has been seldom used with Mediterranean freshwater fish. We tagged over 2500 individuals of two threatened cyprinids (Mediterranean barbel *Barbus meridionalis* and Catalan chub *Squalius laietanus*) inhabiting an Iberian stream affected by water diversion, and used mark-recapture methods to evaluate the effects of flow, temperature, and depth on their apparent survival and abundance at hydrologically-altered and perennial reaches. Eighteen percent of barbel and 27% of chub were recaptured at least once and many were recaptured on several occasions. Our results show clear negative impacts of water diversion on fish populations and increased survival in deeper water and at lower temperatures. The lower reaches with permanent flow had higher survival and abundance, whereas the opposite was true for the intermittent reach. Both species studied have life-cycle characteristics that may allow their populations to recover quickly if the natural streamflow were restored, by conserving water and illegal water diversion.

1. Background

Freshwater fish living in Mediterranean climates are well adapted to natural environmental disturbances associated with seasonal shortage in rainfall and consequent flow reduction (Gasith & Resh 1999). Nonetheless, the high population density of the Mediterranean basin causes strong anthropogenic pressure on aquatic ecosystems, with direct and indirect consequences on water quality and quantity (Sabater & Tockner 2010; García-Ruiz et al. 2011). The need for water resources to support industry, hydropower generation, agriculture and domestic use has increased in the last decades, leading to increasing groundwater pumping, surface-water diversions, and construction of dams. These increasing perturbations have aggravated the harsh natural setting for Mediterranean freshwater biota, especially fish, whose survival and life cycle are strongly dependent on water flow (Aparicio et al. 2000; Poff & Zimmermann 2010).

Several ecological studies have already defined the effects of water scarcity on stream fish assemblages in Mediterranean regions, particularly on their density, biomass and species composition (e.g. Pires et al. 1999; Magalhães et al. 2007; Benejam et al. 2010; 2016). Less information is available, however, about how hydrologic alteration affects these fish, especially the cyprinids, at individual and population levels. Cyprinids are the most speciose fish family in European fresh waters (Reyjol et al. 2007) and include many endangered endemic species (Smith & Darwall 2006).

To date there is little information on the survival probability of Mediterranean freshwater fish or how it is affected by anthropogenic disturbances. Mark-recapture methods have been previously used in Mediterranean regions to study fish movement (e.g. Aparicio & de Sostoa 1999; Pires et al. 2014), but these methods are also an essential tool for estimating natural mortality (or survival) in fish stocks (Vetter 1988; Bestgen et al. 2007; Zelasko et al. 2010).

In this study, we explored the effects of water diversion on populations of two threatened cyprinid species: the Mediterranean barbel *Barbus meridionalis* Risso, 1827 and the Catalan chub *Squalius laietanus* Doadrio et al., 2007. We studied these effects in a Mediterranean stream with altered hydrologic and thermal regimes due to water diversion and groundwater pumping (Benejam et al. 2010; Bae et al. 2016). These anthropogenic alterations have detrimental effects on the fish assemblage in the whole basin (Benejam et al. 2008, 2010; Mas-Martí et al. 2010). Here, we assessed the effects of drying and other associated environmental variables on fish survival and abundance using a mark-recapture study. We modelled fish survival probabilities in several reaches where groundwater pumping and diversions have caused intermittent flow, and compared these to survival in reaches with natural perennial flow, using data from a one-year study involving over 2500 individually tagged fish. We hypothesized that stronger effects would occur during the dry season on fish survival in reaches affected by water diversion.

2. Methods

2.1 Fish sampling and marking

Fish were sampled at 10 sites of the Tordera Stream (see Methodology section for more details) with different flow regimes, approximately 1-5 km from each other (Fig. 1). Sites S1 to S3 (hereafter, “stratum 1” or “control”) were located in the upper part of the Tordera Stream mainstem and were characterized by scarcely anthropized habitat, fast-flowing water, clean cobble substratum and dense riparian canopy cover. During

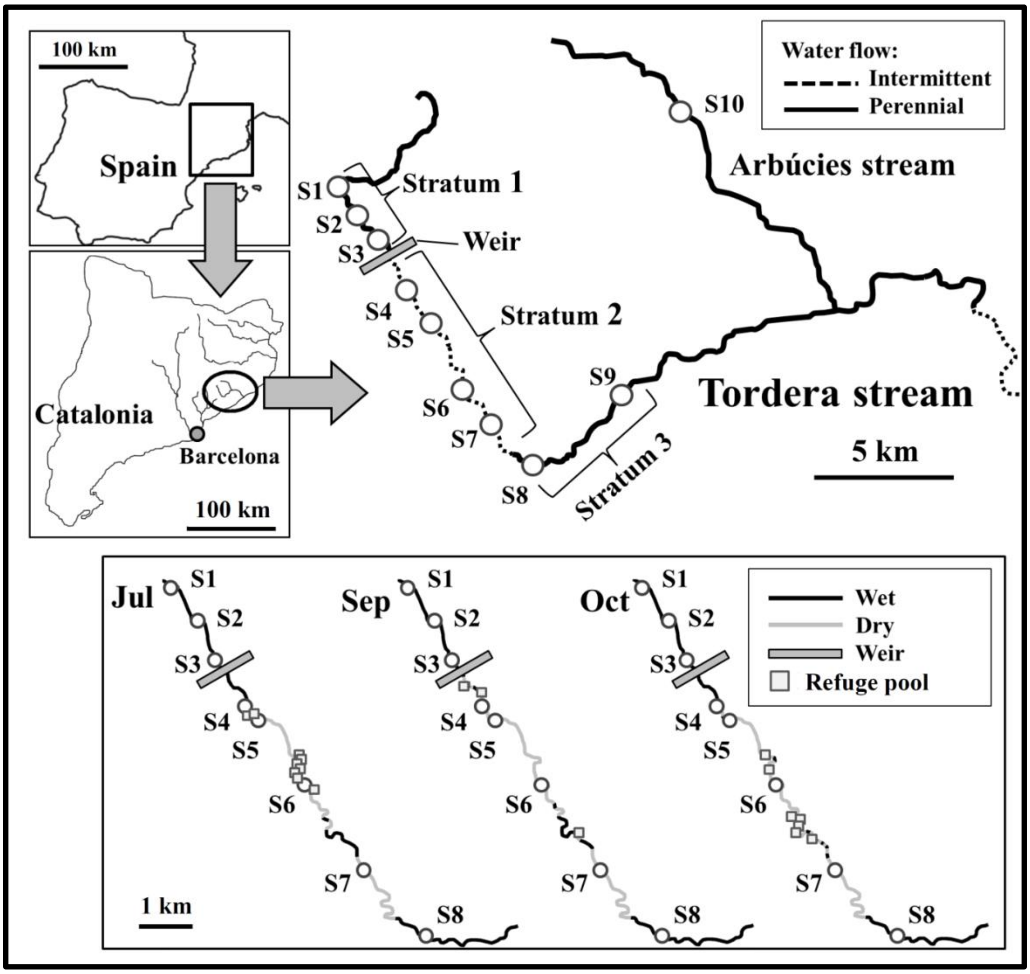


Fig. 1. Location and map of the study area in Tordera Stream and its tributary Arbúcies. Sites 4-7 are intermittent due to water abstraction whereas the rest have permanent flow regimes. Dry reaches and fish refuge pools along stratum 2 during the study period (July, September and October 2012) are also shown.

summer, a weir and diversion canal between sites S3 and S4 extracted about 0.09 m^3 s^{-1} of water for irrigation and urban use, whereas water flow downstream of the weir during the study period ranged 0.09–0.95 m^3 s^{-1} (average: 0.31 m^3 s^{-1}; Bae et al. 2016). When the stream was flowing, the weir was the only obstacle to fish movement along the study segment. However, during summer most of the water was diverted into the canal and the reach downstream became a series of isolated pools separated by long reaches of dry streambed (Benejam et al. 2010; Bae et al. 2016). Sites S4 to S7 (hereafter "stratum 2" or "impact") were located in this intermittent reach. Some of the fish living in the impact reach apparently survived the dry season by moving to a few refuge pools.

About 1 km upstream of S8, the flow was restored by the effluent of a wastewater treatment plant, making the flow regime in S8 and S9 perennial (“stratum 3” or “recovery”). Finally, site S10 was located in the middle part of the Arbúcies Stream, one of the main tributaries of the Tordera basin. We included S10 as an additional control site because it has a perennial flow regime and is affected little by human activities along its course.

Fish were sampled every two months from May 2012 to June 2013 (eight sample dates total) with two different gears. Single-pass electrofishing was conducted in 100-m reaches at each of the 10 sites (Smith-Root LR-24 backpack electrofisher, 100-150 V, 0.8-1.5 A, fully rectified triphasic DC). Fyke nets (10-mm mesh; hereafter, “traps”) were used only at sites S8, S9, S10, because preliminary pilot sampling resulted in too few captures at the other sites. The traps were placed in the same 100-m reaches, within riparian cover (e.g. tree roots, herbaceous plants), because these habitats showed the highest captures in pilot sampling.

For the first marking session in mid-May 2012, we captured fish using only electrofishing at all sites. From the second session (late May) onward, we followed the Cormack-Jolly-Seber design (White & Burnham 1999) at sites S1-S7 where fish were captured once per session via electrofishing. In contrast, at sites S8-S10, where both electrofishing and traps were used, we followed Pollock’s robust design (Pollock 1982; Pollock et al. 1990). At these sites, each primary session contained multiple occasions. Sampling sessions between May-October 2012 contained 4 occasions, whereas those between November 2012-June2013 contained only 2 occasions. In both cases, a single electrofishing pass was conducted on the first occasion and trapping was employed on the remaining days.

All barbel and chub captured were anesthetized with 5-10 drops of clove oil (Aura Cacia® pure essential oil) diluted in 10 L of water, measured to the nearest mm (fork length) and weighed to the nearest 0.1 g. On the first sampling session in mid-May, fish ≥70 mm received a 9-mm Passive Integrated Transponder (PIT) tag (Biomark®) inserted with a pistol implanter into the body cavity, just posterior to the pectoral fin tip. In the following sessions, fish ≥70 mm were scanned for the presence of PIT tags. All unmarked fish ≥70 mm were tagged during subsequent sessions until all PIT tags were used, which occurred during the fourth primary session in October 2012. Individuals <70 mm were not tagged, and all captured fish were released in the middle of the sampling reach following complete recovery.

Open-population mark-recapture models rely on several assumptions to assure unbiased estimates of apparent survival probabilities. Specifically we assumed: i) no tag loss, ii) no marking effect on survival, iii) all emigration is permanent, and iv) no unmodeled heterogeneity in capture probabilities (Pollock et al. 1990). Tag retention and mortality due to marking were estimated through a short-term experiment. One hundred fish (78 barbel and 22 chub) were anesthetized, measured and weighed at an additional site between S8 and S9 on October 12, 2012. One half of the individuals of each species was marked with PIT tags; the second half was anesthetized, measured and weighed only, and used as a control. All the fish were randomly assigned to one of six 0.5-m^2 enclosures (16-17 individuals each) in the stream for 24 hours. Long-term mark retention was further tested at S8 by double marking in the first session, using coded wire tags (CWT; Northwest Marine Technology Inc.) in addition to PIT tags. Coded wire tags (diameter 0.25 mm; length 1.1 mm) were injected hypodermically between the dorsal fin and the lateral line and used as a batch mark. Recaptured fish without PIT tags were scanned with a handheld wand metal detector for CWT presence, which indicated that the PIT tag had been lost. Estimating CWT loss was not possible, since the wand detected both PIT tags and CWT indiscriminately.

Since no refuge pools persisted in summer within the sampling reaches, we decided to sample fish from one of the few easily accessible pools found between S5 and S6 on August 1, 2012, to check for marked individuals. This allowed us to explore the ability of fish to move to refugia during the dry season and perhaps move back to the original site later (i.e., temporary emigration). In addition, 100 unmarked barbel were captured at the pool, marked with PIT tags and released. Chub were not tagged because of their very low density.

2.2 Habitat measurements

Water temperature (*T*; ºC) was measured hourly at one site in each stratum and at site S10 using data loggers (HOBO Water Temp Pro v2, Onset Computer Cooperation, Boume, MA). Water flow (Q; m^3 s^{-1}) was also measured hourly with calibrated data loggers (Heron Instruments dipperLog and Solinst® 3001 Levelogger® Edge, Ontario, Canada) in strata 1 and 2 and site S10, and provided on a daily basis from stratum 3 by the Catalan Water Agency (data available at http://www.gencat.cat/aca). The temperature and flow data during the study are thoroughly analyzed elsewhere (Bae et al. 2016). Maximum depth (cm) across transects perpendicular to flow was measured every 10 meters along the sampling reach, at all sites for each session (Table 1). For our analysis we defined categories for temperature and flow: defining low, intermediate and high temperatures ($T < 10$ ºC, 10-15 ºC, and $T > 15$ ºC respectively) as well as low, intermediate and high flows ($Q < 0.05$ m s^{-1}, 0.05-0.25 m s^{-1}, and $Q > 0.25$ m s^{-1}, respectively), when the reach was not dry.

On July 24th, September 5th and October 7th, 2012 the spatial extent of water was mapped in the intermittent reaches, following Schlosser (1995) and Labbe and Fausch (2000). On these dates, we walked the entire length of stratum 2, recording with a GPS the UTM coordinates of refuge pools and the starting and ending points of all wet segments, which were then mapped (Fig. 1).

Table 1. Mean (minimum-maximum) water depth measured at 10 sampling sites in the Tordera (S1-S9) and Arbúcies Stream (S10) during the study period. See text for details.

Site	Mean width (m)	Mean depth (cm)
S1	6.35 (5.24 - 6.97)	56.6 (49.1 - 65.9)
S2	6.82 (6.17 - 7.24)	60.81 (54.8 - 73.2)
S3	5.87 (4.95 - 6.89)	40.52 (31.9 - 53.2)
S4	5.32 (3.84 - 7.36)	35.99 (25.2 - 44.6)
S5	4.98 (2.7 - 5.83)	29.98 (26 - 35.6)
S6	6.53 (5.2 - 8.56)	29.8 (22.3 - 36.6)
S7	6.06 (5.7 - 7.09)	26.42 (21.9 - 29.9)
S8	7.28 (5.05 - 11.94)	41.67 (31.5 - 54.9)
S9	7.32 (6.02 - 9.87)	50.73 (39.6 - 61.8)
S10	6.32 (4.21 - 7.1)	32.51 (25.28 - 43.8)

2.3 Statistical modeling

We developed two datasets to explore variation in survival probability among intermittent and permanent reaches: the first dataset included a single occasion per session (sites S1-S7, Cormack-Jolly Seber design [CJS]), and the second dataset included data from sites with multiple occasions during each primary session (S8-S10, robust design [RD]).

A preliminary step in the analysis was to test the possible effect of fish fork length (FL) on either initial (*p*) or recapture (*c*) probabilities. Fish size is known to affect catchability (e.g. Bohlin et al. 1989; Benejam et al. 2012), at least by electrofishing (Reynolds 1983; Riley & Fausch 1992; Benejam et al. 2012). Individual covariates like FL vary through time; therefore, knowing or estimating FL for each individual (recaptured or not), during each session of the study would be necessary to include this covariate in an open-population model (e.g., CJS or RD model). Instead, we tested the effect of FL on capture probabilities using the closed-population Huggins estimator applied to each session that had four secondary occasions (Huggins 1989, 1991; Alho 1990). The Huggins model allows including individual covariates like FL since the time span of the sampling is short enough to assume that the population is closed and that growth is minimal (e.g., a few days). We separately analyzed data from the three primary sessions with four secondary occasions (late May, July and October 2012) at sites S8, S9, S10, and assumed that the populations were closed during each session. In addition to FL, other factors potentially affecting capture probabilities were included in the models: fish species, site, fishing gear (electrofishing and traps may have different effectiveness), and trap response (e.g. fish may learn to avoid traps, thus $p \neq c$) effects.

For all Huggins, CJS and RD models, we considered all possible additive combinations of factors which we hypothesized to influence model parameters. Results from these preliminary analysis suggest that FL and trap response had little influence on capture probability (see the “Results” section) and were thus ignored in subsequent analyses, but fish species, site and gear type were retained when appropriate.

Individuals captured at sites included in the CJS design were grouped and analyzed by stratum (1 and 2), aggregating the low number of recaptures within each stratum to provide larger sample sizes. In this study, our main goal was to compare apparent survival probabilities (S) among sampling sites with different hydrological and temperature regimes. Accordingly, survival structures included all additive combinations of the variables that were suspected to affect survival (S): stratum, session, temperature, depth and flow. Capture probability (p) was modeled with stratum, temperature, depth and flow. Species and gear were not included since only barbel was abundant enough for estimation, and trapping was not carried out in strata 1 and 2.

Pollock’s robust design model (Pollock 1982; Kendall et al. 1997) allows estimating several demographic parameters, including: apparent survival probability between two sampling sessions i and j (S_i); capture and recapture probabilities for secondary occasions j in session i (p_{ij} and c_{ij}, respectively); probability of temporarily emigrating between sessions i and j and thus being unavailable for capture during a session j

(temporary emigration parameters, γ''_i and γ'_i); and population abundance during session i (N_i).

Model structures for survival analysis included all additive combinations of the following variables: fish species, site, species and site interactions, session, temperature, flow and depth. Temperature was expected to potentially affect fish survival especially in summer (Magoulick & Kobza 2003), whereas flow and depth are direct indices of hydrological disturbance and presence of refugia, respectively. Parameters related to temporary emigration were not estimated (we fixed $\gamma''_i = \gamma'_i = 0$), because during this study no individuals were recaptured at sites other than where they were marked.

To account for temporal and spatial variation in capture probabilities, we included factors known to affect p based on our closed-population analysis (fish species, gear type) and also explored the effects of temperature, flow and depth. Temperature may affect efficiency of traps, since cold temperatures reduce fish movement, and also may affect electrofishing, as fish tend to move into deep pools during winter (Hall 1972; Gowan & Fausch 1996; Albanese et al. 2004). Depth and flow were expected to affect electrofishing, because dispersion of electricity in more or deeper water reduces catchability (Reynolds 1983). These two variables express the same kind of variation across sites and sessions, so we fit models with just one of these variables at a time. The same was done with temperature and flow, since both vary through time, and were expected to contribute to observed variation in a similar way. The Arbúcies stream has a higher flow than the Tordera mainstem (respective mean discharge during the study period: 0.29 versus 0.14 $m^3 s^{-1}$), so the intervals were defined by different values, but the categories matched those for the permanent-flow segments of Tordera Stream (strata 1 and 3).

For analyses of both RD and CJS models, the candidate models were evaluated following the stepwise procedure described in Zelasko et al. (2010): first, using the most general survival structure (RD analysis: $S(t + \text{site} + \text{species} + \text{site} \times \text{species})$; CJS analysis: $S(t + \text{stratum})$), we obtained the most parsimonious model structure for capture probability. Retaining the best supported capture probability (p) structure, we explored all survival model structures (all possible additive combinations of factors/variable hypothesized to influence survival). Model selection was evaluated with Akaike's information criterion adjusted for small sample sizes (AIC_c; Burnham & Anderson 1998).

3. Results

3.1 Fish marking

A total of 2030 barbel and 374 chub were marked across the 10 sampling sites during the study. Barbel was the most widespread and abundant native fish species in the Tordera, and was found at all the sampling sites. In contrast, chub had a smaller range, and was only abundant at sites S8-S9, with individuals captured occasionally at the other sites. Three hundred fifty-six barbel were recaptured at least once, and 270 were captured in multiple sessions, but only 63 were recaptured in 3 or more sessions. Likewise, of the 101 chub that were recaptured at least once, 93 were captured in multiple sessions, but only 14 were recaptured in 3 or more sessions. Mean fork length of marked barbel was 112 mm, with a maximum of 213 mm. Mean FL of marked chub was 145 mm, with a maximum of 297 mm.

No fish died during the short-term experiment designed to measure tag-induced mortality and mark retention. These fish were released in apparently healthy condition. All 39 marked barbel retained their PIT-tag during the 24 hours of the experiment, whereas one of the 11 chub lost its tag. At site S8, 135 barbel and 87 chub received a double mark in mid-May 2012, with PIT tags and CWT, to determine long-term retention. Of these, 32 barbel and 18 chub were recaptured at least once, of which only one barbel had lost the PIT-tag. Only 2% (1 of 50 in both cases) of the tags were lost both in the short-term and in the long-term tag retention tests.

3.2 Selection of factors affecting capture probability

Twelve Huggins models were fit to data from each of the 3 primary sessions with 4 secondary occasions (May, July and October 2012) to test the effect of FL and several environmental variables on capture probability. The top-ranked structures were slightly different for the three sessions (Table 2) but the models accounting for 95% of model weight generally included the effects of species, site, gear (electrofishing or trapping) and trap response (Table 2, Table 3). The effects of fish species, site, and gear varied markedly with season, often changing in sign, but the estimated effect of fish fork length was negligible (i.e., at or near zero, Table 3).

Given these results, the capture probability structures of RD models were built including gear, species and site as sources of variation. A trap-response effect was excluded because its effect was confounded with gear effects, especially for the sessions with only 2 occasions: i.e., within each primary session it was possible to recapture a fish only by trapping, because electrofishing was conducted during the first occasion only. We considered gear type more likely to affect capture probability and chose to include this variable instead of a trap response, because electrofishing and trapping work in different ways and were expected to have different effectiveness. Moreover, trap-response effects are known to affect precision but do not produce bias for mark-recapture estimates of survival (Nichols et al. 1984).

3.3 Fish survival probability and abundance

To explore factors influencing apparent survival, we fitted 14 Cormack-Jolly-Seber (Table 4, Table 5) and 37 robust design models (Table 6, Table 7) to the data sets for strata 1-2 and for sites S8-S10, respectively.

The best CJS model carried approximately 50% of the AIC_c weight and the second-best model was also well supported ($\Delta AIC_c < 1$). Due to the uncertainty between the two top-ranked models, model-averaged estimates were employed (Table 8). Both models suggested that depth influenced apparent survival probability and water flow influenced capture probability. The top model suggested that apparent survival was also influenced by temperature.

The best supported RD model (AIC_c weight = 0.79) suggested that apparent survival probability varied among sites, species and sessions (*t*) (Table 6). This model was 4 times more likely than the second best model, which also included an interaction between site and species for apparent survival (AIC_c weight = 0.18) and these were the only models with AIC_c weight > 0.01 (Table 6). Capture probability varied with species, fishing gear, temperature and depth.

Overall, survival probabilities varied among species, sites, sessions, temperature and depth (Table 8). Estimates where higher for chub (Table 8, Fig. 2) at the two sites where the species coexisted (S8, S9). As expected, monthly survival was lower at impacted sites (stratum 2), i.e. about half as much as at stratum 1, according to CJS models (stratum-2 monthly survival, May-Jul 2012: 0.36 ± 0.12 SE, Jul-Oct: 0.47 ± 0.15 SE; stratum 1, May-Jul 2012: 0.82 ± 0.12 SE, Jul-Oct: 0.85 ± 0.10 SE) (Fig. 3). The difference between the two strata is much higher if we consider the whole dry period, about three months, instead of comparing monthly survival probability, i.e. the survival probability at stratum 2 from july to september at stratum 1 was about 0.82 × 0.85 ×

0.85 = 0.57, whereas at stratum 2 it was about 0.37 × 0.47 × 0.47 = 0.08, about one order of magnitude lower.

Survival probabilities were highest and more constant at S10, whereas at S8 and S9 they were more variable throughout the year. Survival was the highest between July and November 2012, and between January and April 2013 (Table 8, Fig. 2). At strata 1 and 2, apparent survival was higher during periods with low temperatures and deep water (Table 8). Capture probabilities varied among species, gear, flow, temperature and depth. Capture probabilities of both species were generally low, but higher for barbel than chub and also better when using electrofishing compared to trapping gears (Table 8, Fig. 2). Estimates were higher at S8, S9 and S10: using electrofishing, the highest capture probabilities were around 0.35 at Site 9 between spring and summer of both years, when temperatures were higher (Table 8, Fig. 2). In contrast, capture probabilities were always less than 0.07 in strata 1 and 2, with little variation among sessions. There was a strong negative effect of high flows to capture probability, especially at stratum 1, where water was deeper (Table 8).

Barbel relative abundance was much higher in stratum 3 (sites S8 and S9) and at S10 than at the other sites. The average barbel catch per electrofishing pass was 6.5 individuals in stratum 1, 3.4 in stratum 2, 25.0 in stratum 3 and 110.6 at S10. From May to October 2012, estimated densities of both species (Table 9) were similar at S8 and S9, though highly variable among sessions. Few barbel were caught in July 2012 during the four secondary sampling occasions at S8, by electrofishing or by traps. In contrast, at S10 barbel abundance was highest in July (> 1.2 individuals m^{-2}). Given the few recaptures after October 2012 and no releases of newly marked individuals, abundance estimates were not possible.

(*Next page*)

Table 2. Model selection results for Huggins models fit to mark-recapture data from 3 sites (S8-S10) during 3 closed-population sessions (May, July, and October). Factors included: fishing gear type (gear), effect of fish fork length on electrofishing (FL_E) and trapping (FL_T) catchability, trap-response, fish species (species), and sampling site (site). $p(.)$ indicates constant capture probability across all occasions and sites. The models are ordered by Akaike's Information Criterion adjusted for small sample size bias (AIC_c), ΔAIC_c, and AIC_c weight (i.e., the support for the model in question relative to the entire set of candidate models). Also given is the number of parameters in each model (K) and model deviance (-2 × log-likelihood of the model).

May	Deviance	AIC$_c$	Δ AIC$_c$	AIC$_c$ Weights
p(FL$_{E,T}$ + gear + species + site)	2135.4	2149.5	0.00	0.91
p(FL$_E$ + gear + species + site)	2143.4	2155.4	5.97	0.05
p(FL$_E$ + gear + trap response + species + site)	2142.6	2156.6	7.17	0.03
p(gear + species + site)	2148.3	2158.4	8.87	0.01
p(gear + trap response + species + site)	2147.7	2159.8	10.31	0.01
p(FL$_E$ + gear)	2222.7	2228.8	79.27	0.00
p(FL$_{E,T}$ + gear)	2221.2	2229.3	79.77	0.00
p(FL$_E$ + gear + trap response)	2221.4	2229.4	79.95	0.00
p(gear)	2228.8	2232.8	83.38	0.00
p(gear + trap response)	2227.5	2233.5	84.06	0.00
p(.)	2292.9	2294.9	145.41	0.00
p(species + site)	2387.4	2393.4	243.94	0.00
July				
p(gear + trap response + species + site)	786.4	798.4	0.00	0.53
p(FL$_E$ + gear + species + site)	786.3	800.3	1.92	0.20
p(gear + trap response)	794.5	800.5	2.05	0.19
p(FL$_E$ + gear + trap response)	794.4	802.5	4.04	0.07
p(gear + species + site)	805.6	815.6	17.23	0.00
p(FL$_E$ + gear + trap response + species + site)	805.6	817.7	19.24	0.00
p(FL$_{E,T}$ + gear + species + site)	805.2	819.2	20.80	0.00
p(.)	819.8	821.8	23.41	0.00
p(gear)	819.4	823.4	24.97	0.00
p(FL$_E$ + gear)	819.4	825.4	26.97	0.00
p(FL$_{E,T}$ + gear)	818.3	826.4	27.93	0.00
p(species + site)	858.7	864.7	66.26	0.00
October				
p(FL$_E$ + gear + species + site)	758.9	773.0	0.00	0.53
p(gear + trap response + species + site)	761.3	773.3	0.30	0.45
p(gear + trap response)	774.1	780.2	7.13	0.01
p(FL$_E$ + gear + trap response)	773.7	781.7	8.67	0.01
p(gear)	804.6	808.6	35.56	0.00
p(FL$_E$ + gear)	804.1	810.1	37.10	0.00
p(gear + species + site)	800.9	810.9	37.93	0.00
p(FL$_{E,T}$ + gear)	804.0	812.0	39.02	0.00
p(FL$_E$ + gear + trap response + species + site)	800.6	812.6	39.58	0.00
p(FL$_{E,T}$ + gear + species + site)	798.8	812.9	39.86	0.00
p(.)	1039.6	1041.6	268.54	0.00
p(species + site)	1062.5	1068.5	295.44	0.00

Table 3. Estimated effects of different factors on capture (p) and recapture (c) probability, and using the best supported Huggins models (the two best-supported models were used for October, due to high uncertainty, see text and Table 2). Factors included: fishing gear type (gear), effect of fish fork length on electrofishing (FL_E) and trapping (FL_T) catchability, trap-response, fish species (species), and sampling site (site).

		95% Confidence Interval	
May	Estimate	Lower	Upper
***p*($FL_{E,T}$ + gear + species + site)** AIC$_c$ weight = 0.91			
Gear: electrofishing	-3.36	-4.47	-2.24
Gear: traps	-3.32	-4.35	-2.28
FL_E	0.00	-0.01	0.00
FL_T	-0.01	-0.02	0.00
Species (Barbel)	1.44	0.77	2.10
Site: S8	0.40	-0.27	1.06
Site: S9	1.92	1.38	2.47
July			
***p*(gear + trap response + species + site)** AIC$_c$ weight = 0.53			
Gear: electrofishing	-1.69	-2.48	-0.91
Gear: traps (*p*)	-0.83	-1.74	0.08
Recapture probability (*c*): traps only	-2.92	-3.71	-2.14
Species (Barbel)	0.71	0.03	1.40
Site: S8	1.29	0.36	2.23
Site: S9	0.38	-0.11	0.88
October			
Model 1: *p*(FL_E + gear + species + site) AIC$_c$ weight = 0.53			
Gear: electrofishing	2.81	1.49	4.13
Gear: traps (*p*)	1.86	0.93	2.78
Recapture probability (*c*): traps only	-2.05	-2.78	-1.32
FL_E	-0.01	-0.01	0.00
Species (Barbel)	-1.10	-1.77	-0.43
Site: S8	-0.70	-1.20	-0.20
Site: S9	-0.65	-1.30	0.00
Model 2: *p*(gear + trap response + species + site) AIC$_c$ weight = 0.45			
Gear: electrofishing	1.98	1.21	2.75
Gear: traps (*p*)	1.72	0.80	2.63
Recapture probability (*c*) : traps only	-2.14	-2.87	-1.41
Species (Barbel)	-0.94	-1.59	-0.29
Site: S8	-0.71	-1.21	-0.22
Site: S9	-0.71	-1.36	-0.06

Table 4. Model selection results for Cormack–Jolly–Seber models fit to mark-recapture data of Mediterranean barbel in strata 1 and 2. Survival structures are shown, fit using the best capture probability structure (*p*(*Q*)). See Table 5 for *p* structures. Models include different structures for apparent survival (*S*) modeled as a function of session (*t*), stratum, temperature (*T*), depth (*D*) and flow (*Q*). *S*(.) indicates constant survival across sessions and strata. The models are ordered by Akaike's Information Criterion adjusted for small sample size bias (AIC_c), ΔAIC_c, and AIC_c weight (i.e., the support for the model in question relative to the entire set of candidate models). Also given are the number of parameters in each model (*K*) and model deviance (-2 × log-likelihood of the model).

Model	AIC_c	ΔAIC_c	AIC_c weight	*K*	Deviance
S(*T* + *D*)	288.1	0.00	0.48	6	61.38
S(*D*)	288.8	0.66	0.34	5	64.09
S(stratum)	291.6	3.48	0.08	5	66.91
S(*t* + stratum)	293.3	5.23	0.03	11	56.25
S(*t*)	294.0	5.92	0.02	6	67.30
S(*Q*)	302.7	14.58	0.00	6	75.96
S(*T*)	304.3	16.22	0.00	5	79.65
S(.)	311.0	22.87	0.00	4	88.33

Table 5. Model selection results for Cormack–Jolly–Seber models fit to mark-recapture data of Mediterranean barbel in strata 1 and 2. Models include different structures for capture probability (*p*) modeled as a function of stratum, temperature (*T*), depth (*D*) and flow (*Q*), using the most general survival structure (*S*(*t*+ stratum)). *p*(.) indicates constant capture probability across sessions and strata. The models are ordered by Akaike's Information Criterion adjusted for small sample size bias (AIC_c), ΔAIC_c, and AIC_c weight (i.e., the support for the model in question relative to the entire set of candidate models). Also given is the number of parameters in each model (*K*) and model deviance (-2 × log-likelihood of the model). See text for more details.

Model	AIC_c	ΔAIC_c	AIC_c weight	*K*	Deviance
p(*Q*)	293.3	5.23	0.03	11	56.25
p(*T* + *D*)	294.5	6.45	0.02	11	57.47
p(*D*)	295.9	7.85	0.01	10	60.96
p(*T*)	296.2	8.10	0.01	10	61.21
p(.)	299.0	10.90	0.00	9	66.09
p(stratum)	299.9	11.85	0.00	10	64.96

Table 6. Robust design models used to estimate apparent survival (S) and abundance of Mediterranean barbel and Catalan chub at sites S8-S9-S10. Survival structures are shown, fit using the best capture probability structure (*p*(species + gear + T + D)). See Table 7 for *p* structures. The models are ordered by Akaike's information criterion adjusted for small sample size bias (AIC$_c$). See Table 4 for other abbreviations. Effects included: time (*t*), site, species, temperature (*T*), depth (*D*), and flow (*Q*). *S*(.) as in Table 4.

Model	AIC$_c$	ΔAIC$_c$	AIC$_c$ Weight	*K*	Deviance
S(*t* + site + species)	7478.4	0.00	0.79	16	12621.3
S(*t* + site + species + site × species)	7481.4	2.97	0.18	18	12620.2
S(*t* + site + species + site × species + *T* + *D*)	7486.4	7.94	0.01	21	12619.1
S(*t* + site)	7527.1	48.66	0.00	15	12672.0
S(*t* + species)	7529.8	51.34	0.00	13	12678.7
S(*t* + species)	7531.8	53.37	0.00	14	12678.7
S(species + *T* + *D*)	7554.5	76.08	0.00	11	12707.5
S(*t*)	7558.1	79.69	0.00	13	12707.1
S(*T* + *D*)	7624.8	146.41	0.00	10	12779.8
S(site + species)	7629.2	150.79	0.00	10	12784.2
S(species + *T*)	7650.1	171.66	0.00	10	12805.1
S(species + *D*)	7659.4	180.91	0.00	9	12816.4
S(species + *Q*)	7670.1	191.65	0.00	11	12823.1
S(*T*)	7675.7	197.22	0.00	9	12832.7
S(site + species + gear + *T* + *D*)	7694.1	215.64	0.00	9	12851.1
S(species)	7697.8	219.31	0.00	8	12856.8
S(species)	7697.8	219.31	0.00	8	12856.8
S(*Q*)	7699.5	221.09	0.00	9	12856.6
S(*D*)	7716.65	238.20	0.00	8	12875.7
S(.)	7726.10	247.64	0.00	7	12887.1

Table 7. Model selection results for robust design models fit to mark-recapture data of Mediterranean barbel at sites S8-9-10. Models include different structures for capture probability (p) modeled as a function of site, temperature (T), depth (D) and flow (Q), using the most general survival structure ($S(t$ + site + species + site × species)). $p(.)$ indicates constant capture probability across sessions and sites. The models are ordered by Akaike's Information Criterion adjusted for small sample size bias (AIC_c). See Table 1 for more abbreviations.

Model	AIC_c	ΔAIC_c	AIC_c Weight	K	Deviance
p(species + gear + T + D)	7481.4	2.97	0.18	18	12620.2
p(gear + T + D)	7531.4	52.93	0.00	17	12672.2
p(species + gear + D)	7532.5	54.04	0.00	16	12675.3
p(species + gear + T)	7575.5	97.09	0.00	17	12716.3
p(gear + T)	7598.2	119.79	0.00	16	12741.1
p(species + gear + Q)	7598.8	120.38	0.00	17	12739.6
p(gear + D)	7600.6	122.20	0.00	15	12745.5
p(species + gear)	7604.6	126.19	0.00	15	12749.5
p(species + D)	7736.16	257.71	0.00	15	12881.0
p(T + D)	7756.59	278.14	0.00	16	12899.4
p(species + T)	7797.80	319.34	0.00	16	12940.6
p(D)	7800.20	321.74	0.00	14	12947.1
p(species + Q)	7801.74	323.29	0.00	16	12944.6
p(species)	7811.65	333.20	0.00	14	12958.6
p(T)	7820.53	342.08	0.00	15	12965.4
p(Q)	7837.77	359.32	0.00	15	12982.6
p(.)	7846.58	368.13	0.00	13	12995.5

Table 8. Estimated effects of different variables on apparent survival (S) and capture probability (*p*), assuming a logit link function, using the best supported Cormack-Jolly-Seber (CJS) and robust design (RD) models. CJS models were used to analyze data sites in strata 1 and 2, whereas RD models were fit to data from sites S8-S10. Effects included: session (*t*), site, species, gear, temperature (*T*), depth (*D*) and flow (*Q*). CJS models included two temperature categories ("High" and "Low"), whereas RD models included three, because "Intermediate" temperatures were never detected during sampling at strata 1 and 2.

			95% Confidence Interval	
	CJS model 1: *S*(*T* + *D*) *p*(*Q*) AIC$_c$ weight = 0.48	Estimate	Lower	Upper
S	Intercept (High *T*)	-0.91	-1.85	0.02
	Low *T*	16.28	-4386.13	4418.70
	Depth	0.04	0.02	0.08
p	Intercept (Intermediate *Q*)	-3.46	-4.10	-2.83
	Low *Q*	0.67	-0.17	1.50
	High *Q*	-43.19	-43.19	-43.19
	CJS model 2: *S*(*D*) *p*(*Q*) AIC$_c$ weight = 0.34			
S	Intercept	-0.88	-1.93	0.17
	Depth	0.07	0.02	0.12
p	Intercept (Intermediate *Q*)	-3.68	-4.40	-2.90
	Low *Q*	0.41	-0.39	1.22
	High *Q*	-17.06	-5531.46	5497.34
	RD model 1: *S*(*t* + site + species) *p*(species + gear + *T* + *D*) AIC$_c$ weight = 0.79			
S	Intercept (session 7, site 10, species: barbel)	1.36	0.60	2.12
	Site 8	-1.06	-1.40	-0.71
	Site 9	-1.03	-1.36	-0.70
	Species: chub	1.26	0.85	1.65
	Session 1	-1.79	-2.65	-0.94
	Session 2	-0.18	-1.02	0.66
	Session 3	1.56	0.33	2.80
	Session 4	1.95	-1.57	5.48
	Session 5	0.20	-1.10	1.51
	Session 6	14.39	-1226.35	1255.13
p = *c*	Intercept (species: barbel; gear: electrofishing; intermediate *T*)	-4.84	-5.51	-4.17
	Species: chub	-1.06	-1.37	-0.75
	Gear: traps	-0.89	-1.01	-0.77
	Low *T*	0.24	-0.25	0.74
	High *T*	1.43	0.95	1.92
	Depth	0.05	0.04	0.05

Table 9. Estimated fish abundance ($\hat{N}$) and density ($\hat{D}$) with standard errors (SE) at sites S8, S9 and S10 from May to October 2012. Areas of the sampling reaches are also shown.

	Site	Session	Sampling area (m^2)	$\hat{N}$ ± SE	$\hat{D}$ ± SE
Barbus meridionalis	S8	Late-May	642	303 ± 25	0.47 ± 0.04
	S8	Jul	505	9 ± 4	0.02 ± 0.01
	S8	Oct	1194	319 ± 35	0.27 ± 0.03
	S9	Late-May	636	223 ± 16	0.35 ± 0.02
	S9	Jul	602	121 ± 14	0.20 ± 0.02
	S9	Oct	659	84 ± 12	0.13 ± 0.02
	S10	Late-May	710	575 ± 47	0.81 ± 0.06
	S10	Jul	421	503 ± 59	1.19 ± 0.14
	S10	Oct	640	526 ± 59	0.82 ± 0.09
Squalius laietanus	S8	Late-May	642	281 ± 51	0.44 ± 0.08
	S8	Jul	505	62 ± 22	0.12 ± 0.04
	S8	Oct	1194	126 ± 37	0.11 ± 0.03
	S9	Late-May	636	192 ± 30	0.30 ± 0.05
	S9	Jul	602	167 ± 35	0.28 ± 0.06
	S9	Oct	659	350 ± 63	0.53 ± 0.09

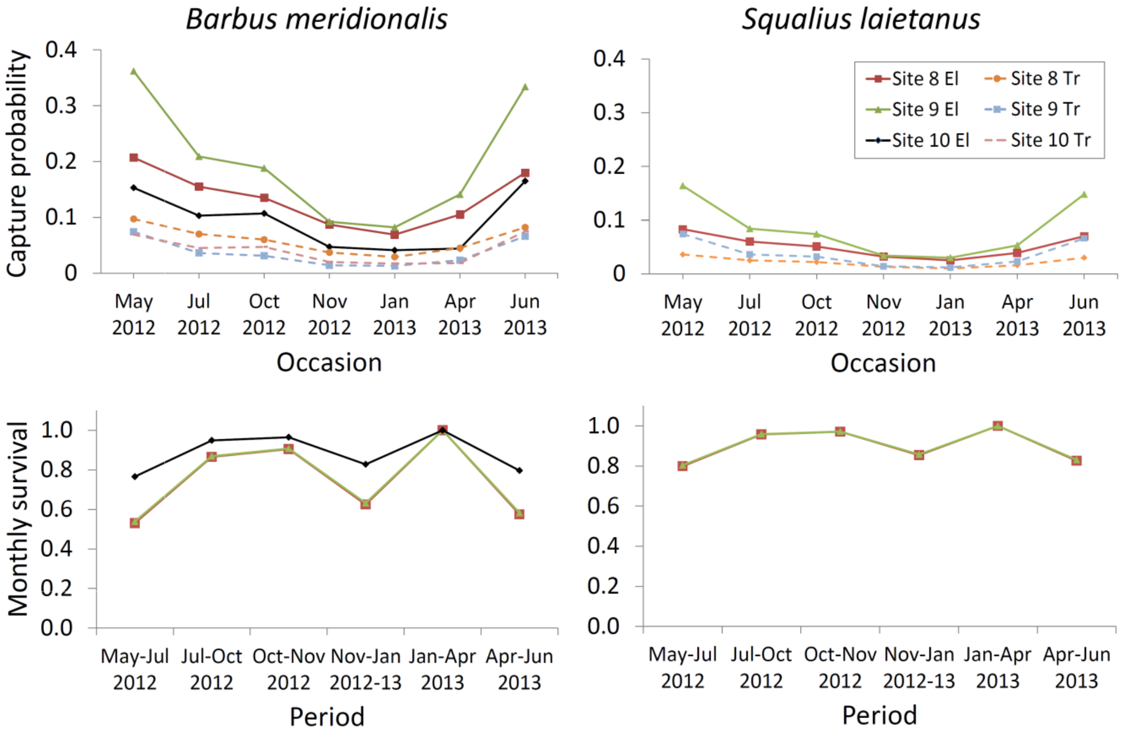


Fig.2. Estimated probability of capture and apparent monthly survival probabilities for Mediterranean barbel *Barbus meridionalis* and Catalan chub *Squalius laietanus* in Tordera Stream at sites S8-S10. The top-ranked RD model estimates are shown. Abbreviations: El = electrofishing; Tr = trapping.

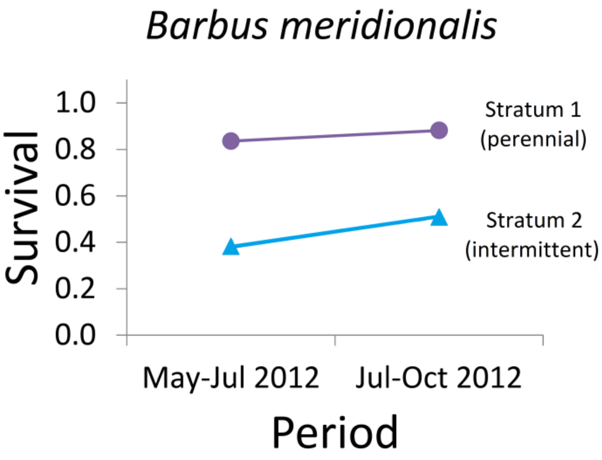


Fig. 3. Estimated monthly apparent survival for Mediterranean barbel *Barbus meridionalis* in strata 1 and 2 of Tordera Stream, resulting from CJS analysis. Average estimates of the two top-ranked models are shown.

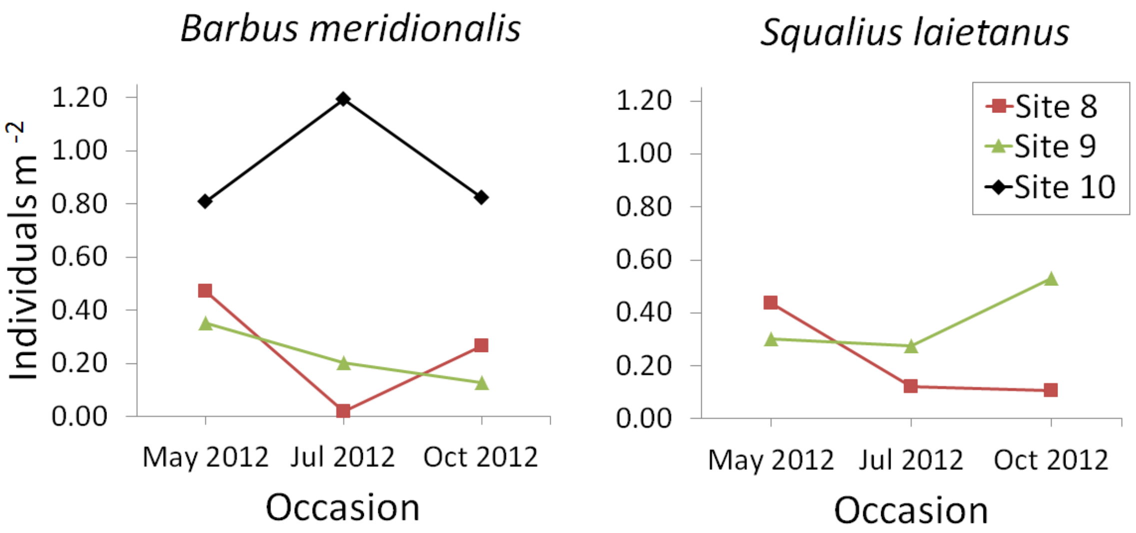


Fig. 4. Density of Mediterranean barbel *Barbus meridionalis* and Catalan chub *Squalius laietanus* at sites S8-S9 (Tordera mainstem) and S10 (Arbúcies Stream), based on abundance estimates from the top-ranked Robust Design model. No chub were captured at S10 during the study period.

4. Discussion

This study highlighted how survival and abundance of two endangered cyprinid species were strongly reduced by human-induced hydrologic alteration. The probability for barbel survival throughout the dry season was an order of magnitude lower in intermittent than in permanent reaches. These findings confirm and complement previous observations in the same basin (Benejam et al. 2008, 2010; Mas-Martí et al. 2010). During the hydrological year 2012-2013 we studied, the seasonal drought in the Tordera catchment was particularly intense and prolonged, because there was little rainfall and persistent water demand. The intermittent reaches (stratum 2) progressively dried up in the first half of July and remained dry until the second half of October, showing the maximum extent of drying at the beginning of October (Fig. 1). This caused the few refuge pools in the stream to be disconnected from each other for about three months. This situation improved temporarily in November because of a discharge peak, but the drought persisted during winter, with no rainy periods that affected flow until March 2013.

The effects of water diversion on the barbel population were strong and statistically significant, but chub was perhaps the most affected of the two species. Although barbel was consistently present at all sampling sites across the watershed, chub was abundant only in the lower stream course (S8-S9); it was almost entirely extirpated from the intermittent stratum 2 in which it was once common (Benejam et al. 2010), and also from the uppermost stratum 1 where the very small subpopulation of chub is now isolated from the main one of the lower river because of the weir that blocks upstream movement. In contrast to barbel, the chub is a water-column dweller, inhabiting only runs and pools, and is likely more affected by reduced water depth due to water diversion. This indicates that the sensitivity to anthropogenic perturbations of Mediterranean barbel may be lower, and the sensitivity of Catalan chub higher, than previously thought (Maceda-Veiga and de Sostoa 2011). The barbel seemed to find the most suitable conditions in S10, where its abundance was high all year long and where it showed the highest and most constant survival during the studied period. Chub has never been found at that site, likely because of the shallow and turbulent riffles that characterize that reach which are poor habitat for this species. Both species were highly abundant in the lower sampling reaches S8-S9, where summer flow is largely supplied by treated wastewater effluents. This observation contrasts with the general belief that

Mediterranean barbel prefers uncontaminated waters (Doadrio et al. 2011; Maceda-Veiga & de Sostoa 2011). Catalan chub seemed to take advantage of water contamination as well, but the consequences of flow alteration were remarkable on this species, accordingly with previous studies (Carol et al., 2006; Blanck et al., 2007; Murphy et al., 2013) which questioned the tolerance of this species to anthropogenic perturbation.

The absence of observed mortality due to tagging and the negligible percentage of tag loss suggested no substantial violation of two fundamental assumptions of mark-recapture studies, i.e. equal survival probability for marked and unmarked individuals, and no tag loss during the study period (Krebs 1999). Barbel survival and abundance were lowest in the intermittent stratum 2 and highest at stratum 3 and S10, matching the assumption that under stable environmental conditions, like in downstream and perennial reaches, fish abundances are higher (Grossman et al. 1998; Matthews 1998; Magalhães et al. 2002, 2007; Keaton et al. 2005).

Apparent mortality estimated by mark-recapture models consists of two components: true mortality and emigration. The reduced survival in drying reaches may depend on three mechanisms. First, drying can kill fish unable to find a refuge. Second, lack of flow leads to harsh abiotic conditions and intensified biotic interactions in refuge pools, enhancing predation by both aquatic and terrestrial predators and strong competition for food (Schlosser 1990; Magoulick & Kobza 2003; Dekar & Magoulick 2013). The third mechanism is related to river morphology. Our CJS analysis showed that variation in water depth (a combination of river bed morphology and water flow) resulted in higher barbel survival probability in stratum 1 (characterized by the presence of several deep pools and permanent flow) relative to stratum 2 (which lacked large pools within the 100-m long reaches). Moreover, the stream channel is narrower in stratum 1, so the riparian canopy shades the entire channel and prevents excessive warming under low flow conditions (Li et al. 1994; Davies & Nelson 1994). Conversely, the river bed is wider and less sheltered in stratum 2 (particularly in the vicinity of S6-S7), which degrades physical habitat via increased water temperatures.

The available options for fish when flow decreased in the stratum-2 sampling reaches were to leave the reach or to die, given the absence of refuge pools. Evidence of fish movement in the Tordera stream is provided by the remarkable variation in abundance of both species measured at stratum 3 and S10 among the three sessions from May to October 2012. Abundance varied in different periods depending on sites and may not be attributed to a single cause, though it might be related to spawning in May (Rodriguez-Ruiz & Granado-Lorencio 1992; Lucas & Batley 1996; Ovidio et al. 2007) and to flow variations (Labbe & Fausch 2000; David & Closs 2002; Albanese et al. 2004); for instance, barbel extremely low density at S8 in July 2012 probably depended on the extremely low flows that we observed there in that session. On the other hand, we observed an increase of barbel and chub abundance, respectively at S8 and S9, in October's session, when flows were still low. More generally, since we did not detect any movement between different sampling reaches, we assume that fish ranged over limited distances, searching for the most suitable habitat (e.g. in terms of food and cover availability or flow) under variable environmental conditions.

We recaptured three barbel in a crowded refuge pool in August 2012 which had been marked in S6, about 300 meters downstream, three months before. This is a direct observation of the potential ability of barbel to escape drying by moving. The same pool was found dry in October, so the hundreds of fish accumulating there in August may have moved again during a temporary rewetting, or if not had died. No fish carcasses were observed, but none of the 100 individuals tagged in that pool in August were recaptured in any reaches. Therefore, either true mortality is extremely high in stratum 2 or apparent mortality is largely made up of emigration from the sampling reaches. While the low apparent survival probability might be attributed to permanent emigration from the reaches, the lack of refuge pools in the stratum-2 reaches, the lack of temporary emigration (i.e. no individuals tagged before drought were recaptured in the intermittent reaches after rewetting) and the failure to document movements between any sampled sites even when the habitat was not disconnected, suggest that the majority of individuals died and that escaping drought to find a refuge did not guarantee survival to barbel in the conditions of severe drying observed during our study.

Temperature varied across seasons and higher summer temperatures corresponded with lower estimated survival of barbel. In spite of this expected result, reliable estimates of survival probability for strata 1 and 2 could be generated only for 2012 (Fig. 3) due to the low number of recaptures after the October 2012 session. At sites S8-9-10, where flow is permanent, the lowest survival estimates for both species did not correspond to the driest period (July-October 2012) (Fig. 2). During the first marking sessions (May 2012) fish were highly abundant; this was a period of rising temperatures and the main spawning peak for European cyprinids. During this period they showed high activity levels compared with other seasons, spending more time in shallow waters and staying away from cover. Thus, their catchability by electrofishing and traps was also high (e.g. Kubecka 1992; Speas et al. 2004; Bodine & Shoup 2010), as confirmed by estimated capture probabilities, which were highest in the spawning periods of both years. The consequence of the decreased catchability after these periods, perhaps together with some emigration of fish from study reaches, may have resulted in decreased apparent survival. Also apparent drops in survival occurred in November 2012-January 2013 and April-June 2013, after corresponding high-flow periods. Fish emigration from sampling reaches could also have been important during these periods, since high rates of fish movement during high flow events have been documented (e.g. David & Closs 2002; Albanese et al. 2004).

Our study shows that water diversion produced strong effects on populations of Mediterranean barbel and Catalan chub in the Tordera catchment. Fish survival probability and abundance were lowest in the reaches where anthropogenic drying occurred. Fish movement may explain a significant fraction of apparent mortality, as suggested by the abundance variation observed during our study at several sites. Nevertheless, the species studied have life-history traits adapted to unstable environments: both achieve sexual maturity in few years (Casals-Martí, unpublished Ph.D. thesis) and might be able to recover rapidly from disturbance (Schlosser 1990; Winemiller & Rose 1992; Labbe & Fausch 2000; Magalhães et al. 2003; Falke et al. 2010a, 2010b), as long as it is not protracted for too many years (Magalhães et al. 2007). Therefore, controlling excessive and illegal water diversion is a critical requirement for fish conservation in the Tordera basin, as well as in many other Mediterranean streams and rivers (Changeaux & Pont 1995; Aparicio et al. 2000; Xenopoulos et al. 2005).

GENERAL DISCUSSION

Global change consequences in the Mediterranean Basin

Freshwater ecosystems are heavily affected by global change around the world (Dynesius and Nilsson, 1994; Levin et al., 2009), and particularly rivers in the Mediterranean basin suffer of a high human pressure and are in a sensitive climate change area (Giorgi, 2006). Mediterranean freshwater ecosystems are threatened by drivers acting at different scales. At the regional scale, and beyond the increase of temperatures and the higher occurrence of heatwave episodes, the Mediterranean basin is expected to experience 20-40% less rainfall than in the last decades, in case of a hypothetic temperature increase of 4-5.5 ºC, after having already decreased of about 20% in the last century (Bates et al., 2008).

At local scale, lesser precipitations, along with an increase of population and land use change, will favor rising water demand, associated to higher surface water diversion, groundwater pumping and dam building (Cooper et al. 2013). This will aggravate the competition between human populations and natural environments for water resources, increasing the frequency and duration of droughts and exceeding the capabilities of river biota to recover afterwards (Gasith and Resh, 1999; Cooper et al., 2013). Land use changes also may lead to increased contaminants inputs. Water scarcity increases contaminants concentrations, especially in streams whose summer water flow is mainly constituted by WWTP effluents, such as many small rivercourses in the Iberian Peninsula (Sabater, 2008). Moreover, the toxicity and bioaccumulation of some classes of pollutants like organophosphates and heavy metals, e.g. in fish, may be favored by higher temperatures (Mc Leod and Pessah, 1973; Murty, 1986). Therefore, the interactions among different drivers of global change on freshwater ecosystems are evident, making hard even distinguishing the effects of stressors of regional and local origin (Ficke et al., 2007).

The Mediterranean Basin is not only a high anthropogenic pressure area, but also a global biodiversity hotspot. In the case of fish, over 250 freshwater fish species have been counted in the area (Smith & Darwall, 2006; Reyjol et al., 2007). Fish have a key ecological role in streams and rivers: feeding on algae, invertebrates and other fish, they affect abundance and species composition of lower trophic levels, influence biogeochemical cycles and even contribute to adjacent terrestrial trophic webs (Baxter et al., 2005). Moreover, fish represent a major part of river biomass. Fisheries provide essential services to humans as a food source and have a recognized value worldwide as economic and recreational resource (Lagler, 1966).

According to the IUCN, the major threats to freshwater fishes in the Mediterranean area, pollution and introduction of nonnative species, followed by drought, water diversion and damming, namely the three main drivers of hydrological alteration and also the ones with the highest increase in the number of species they directly threaten (Smith and Darwall, 2006; Hermoso and Clavero, 2011).

Issues addressed in this thesis

This thesis assessed different kinds of ecological impacts on Mediterranean freshwater ecosystems, directly or indirectly related to hydrological alteration. Our studies encompassed two Iberian watersheds with different histories of human settlements and land use during the last decades: i) the Llobregat, a Pyrenean river flowing across a strongly anthropized area, affected by a wide spectrum of stressors; and ii) the Tordera, a littoral stream whose watershed is moderately affected by human activities, though excessive water demand by local settlements and industry, compared with the naturally modest streamflow, has turned long stream reaches from perennial to artificially intermittent.

Chapters 1, 3 and 4 of this dissertation concern effects of anthropogenic stressors, like pollution and water diversion on Mediterranean fish at population and individual levels. Chapter 2 did not directly regard effects on fish but addressed how water scarcity affects stream water temperature, a fundamental abiotic variable for the entire aquatic ecosystem. In all cases, the observed situations appeared to deviate remarkably from the expected ecological conditions in undisturbed ecosystems.

Consequences of water diversion on fish populations

This thesis has addressed the consequences of water diversion on fish populations in the exemplary case of the Tordera River. Several studies have been conducted to date on this river and its tributaries (e.g. Benejam et al., 2008, 2010; Mas-Martí et al., 2010; Otero et al., 2011; Bae et al., 2016). Chapters 2, 3 and 4 report different aspects of a study providing further knowledge about the stressors the stream ecosystem and especially fish populations have to face. The origin of summer water scarcity in the watershed was identified to be a consequence of land use change in the basin (Benejam et al. 2010), since no decrease in precipitations has been observed in the area in the last decades. Benejam and coworkers fitted the Sacramento soil moisture accounting (SAC–SMA) model to the Tordera rainfall–runoff interaction, revealing that the streamflow is lower and the dry days much more frequent than expected from the amount of precipitation. It was estimated that about 34% of Tordera runoff is diverted for agriculture, industry and urban use. In that study, effects on the fish assemblage were also explored, finding that several fish metrics like number and abundance of sensitive species showed lower values in the intermittent reaches. This thesis complement those findings by addressing effects of water diversion on fish at individual and population levels. The results match our hypotheses in many cases (especially at population level), whereas in some others (especially at individual level) we obtained unexpected relationships.

Effects of dryness on Tordera native cyprinids

We addressed two species in our study, i.e. the Mediterranean barbel *Barbus meridionalis* and the Catalan chub *Squalius laietanus*. We observed the almost total absence of *Squalius laietanus* from the intermittent reaches, and its abundance only in the middle course of the stream. However, the barbel was present throughout the whole basin, though in lower abundance at the intermittent sites.

A moderate barbel density was detected in the intermittent reaches, but populations were apparently decimated by the particularly intense and lasting drought of summer 2012. By contrast, the chub, a species common until few years ago in those reaches (Benejam et al., 2008; 2010), had already been extirpated from there at the beginning of our study. These observations suggests a higher tolerance and resilience of barbel in presence of moderate drought events, compared with the chub, which is likely unable to survive and maintain stable populations under low levels and water depths, in sites submitted to periodic dryness. Barbel appeared well adapted to live in shallow riffles and potentially able to escape drying. In fact, we observed high barbel densities in summer refugia (several hundreds of individuals concentrated in small pools), and directly observed the movement of some tagged individuals recaptured in a pool some hundreds of meters upstream of the drying reach in which they were found.

Survival probability estimates obtained by mark-recapture models confirmed the abundance trends, being lowest in the intermittent reaches. However, the observed ability of fish to actively search for refugia made us believe that the low survival estimates and the drop of fish density observed after rewetting in 2012 may be due in part to emigration and not only to true mortality. Dryness probably killed many individuals, but the survivors in deep pools may be not so few, and after rewetting they might just keep in the same zone, without need of covering long distances to recolonize the original reaches, because of the low competition generated by low fish density (Roberts et al., 2008, Pires et al., 2014). This would be in agreement with the observed high values of barbel growth and, above all, body condition in intermittent reaches (even if not as high as in the middle Tordera reaches): survivors would have a per-capita food available larger than in normal density conditions.

The mechanisms determining higher survival estimations pointed the existence of flow as the fundamental factor for barbel survival, but also to the presence of refuge pools. Temperatures also resulted determinant to describe the survival variability across seasons. Higher temperatures corresponded to lower survival. In the middle Tordera reaches and in the Arbúcies tributary, which represented an additional control site, abundances and survival estimates were the highest. These areas were the best for fish survival: flow permanence, existence of pools, and not excessively high temperatures, coincided in this section.

Despite being usually considered intolerant to low water quality, Mediterranean barbel, as well as chub, showed highest abundances, overall growth rate, annual size increment and body condition in the middle reaches of Tordera, just downstream of the main input of nutrients and organic matter of the watershed, i.e. Santa Maria de Palautordera WWTP (Merseburger et al., 2005; Ortiz et al., 2006). Reasonably, the stable conditions of perennial and more abundant flow in these reaches play an important role for fish survival and wellness (Grossman et al., 1998; Matthews, 1998; Magalhães et al., 2002; 2007; Keaton et al., 2005), but at the same time the high nutrient loads found in these waters appeared to be more beneficial as trophic resources, than detrimental in terms of toxicity, to these cyprinids (e.g. Lappalainen et al., 2001; Beardsley and Britton, 2012) and the other species usually abundant in these reaches, like minnow and eel. Chub body condition seemed to confirm this hypothesis, showing highest values in the middle reaches and gradually decreasing downstream, where no additional nutrient inputs exist (Merseburger et al., 2005). The chub showed the minimum value in the lowermost intermittent reach in the vicinity of the river mouth, where the stress due to water scarcity contributed to chub low condition. This confirmed the lower degree of adaptation of chub to water scarcity in comparison to the barbel.

We can conclude that the variables associated to the heavy alterations of Tordera's hydrological regime affected fish abundance and apparent survival of the population, which were significantly lower in the intermittent reaches. On the other hand, fish growth and condition were not affected by the hydrological alteration, showing the lowest values in the uppermost perennial reaches.

Water diversion and temperature regime in the Tordera Stream

Monthly temperatures, daily temperature variation and air-water temperature relationship followed an upstream-downstream gradient in the Tordera. Temperatures were lower and more constant, both on daily and monthly basis, in the upper than in the lower reaches, as well as air-water temperature slopes were higher downstream, where the influence of groundwater input at constant temperature was lower.

Within these patterns, however, two remarkable anomalies occurred: one was in the reaches affected by water diversion. The alteration of the natural gradients was stronger in in the reach with higher groundwater input, that increased the monthly temperature constancy (i.e. temperature lower in summer and higher in winter, compared with adjacent sites) (Webb, 1992; Caissie, 2006). The site closer to the weir associated to the diversion channel did not show alterations of its temperature regime, since the environment in that site is very similar to the uppermost reaches, with dense canopy cover and forest-dominated contiguous land.

The second anomaly corresponded to the site receiving the WWTP input. Here the air-water temperature slope was higher, likely due to the long time water spends in the shallow, unshaded treatment ponds, which favor the air-water heat exchange. The altered temperature regime was noteworthy in summer, when temperatures were much higher than the adjacent sites.

Temperature is a fundamental ecological variable, which can directly affect the physiology of fish. We observed that fish inhabiting downstream the WWTP input took advantage of the moderate increase of water temperature and nutrient availability. Nonetheless, in a perspective of climate change, the input of waste waters may lead to a further warming of summer water temperatures, with detrimental effects on the stream biota.

Research prospects

Further research steps in the Tordera Stream may be carrying out monitoring of fish populations in the drying reaches, based on the following points:

- a study period encompassing at least one complete dry season, from flow cessation to rewetting;
- a new mark-recapture study, with a sampling design based on contiguous reaches able to detect the among-reaches fish movement, implementing statistical models including temporary and permanent migration, aiming to estimate true mortality; control reaches with the same design; the study reaches must include refuge pools;
- monitoring physical and chemical conditions of refuge pools during the dry season, in order to better understand the physical conditions for fish; assessing the role of stream bed morphology and canopy cover on fish survival and movement.

Trace metal pollution and hydrological alteration effects on fish assemblages

Several aspects need to be taken into consideration to understand the metal pollution effects on fish individuals and assemblages. In general, many sources of variation may contribute to trace element loads in a fish individual. We highlighted significant differences among fish species, which may depend on fish ecological, physiological or behavioral intrinsic traits (Andres et al., 2000; Canli and Atli, 2003; Farkas et al., 2003). Our analyses provided consistent evidence of how smaller fish individuals accumulate, on average, higher metal loads than larger ones. Moreover, we showed that the highest variability occurs at individual level, since specimens of the same species and from the same site can show loads of a same element that differ several orders of magnitude among them. These results suggest that the use of fish in ecotoxicological studies should be based on the analysis of numerous individuals, possibly representative of the fish assemblage species composition and of the population size structure.

No direct relationship necessarily exists between the environmental concentrations of a trace element and its load in a fish tissue: in our study, fish muscle loads did not exactly reflect the contamination gradient existing along the four sampling sites. It is generally assumed, however, that bioaccumulation must show some degree of proportionality with environmental concentrations of a toxic element or compound (e.g.

Andres et al., 2000; Farkas et al., 2003). Water quality in the Llobregat has improved in the last decades by the implementation of numerous WWTPs but contamination persists, especially in the lower river course (Munné et al., 2012). Besides, the Llobregat hydrological regime is heavily altered. Several dams are found in its catchment and about 30% of long-term mean annual flow is diverted for human water uses, so a large part of runoff is constituted by WWTP effluents, particularly during drier years. Since 1945, runoff has decreased of 1-3% per year, in part due to land use change, e.g. forest cover increase because of abandonment of agricultural land, with consequent increased evapotranspiration; on the other hand, temperature increase due to climate change is believed to be responsible of snow cover alteration at the Pyrenees, with effects on Llobregat spring runoff (Marcé et al., 2012). Logically, connections exist among hydrological alteration, water pollution and fish trace metal uptake. Moreover, higher water temperatures, because of climate change, water diversion, WWTP inputs, may aggravate the effects of toxic compounds on fish by increasing the production of bioactivated free radicals which are more toxic than the parent compound (Nemcsók et al., 1987) or by increasing toxin uptake (Murty, 1986) and thus bioaccumulation. Understanding the effects and mitigating the impacts of these multiple components of global change and their interactions is an urgent, challenging task of biodiversity conservation, particularly in Mediterranean fresh waters.

GENERAL CONCLUSIONS

1. Trace metal concentrations in Llobregat fish varied largely with fish species, size and among individuals, chemical elements and sampling sites. Fish size-concentration relationships were negative and significant for most elements in the three most widespread Llobregat fish species (bleak, Languedoc gudgeon and Ebro barbel).
2. High Pb concentrations were found in the Llobregat fish, representing a potential risk for human consumption.
3. Temperature regime in the Tordera was characterized by an upstream-downstream gradient in monthly temperatures, daily temperature variation and air/water temperature relationship. Anomalies were observed at intermittent reaches and at a site affected by a wastewater treatment plant (WWTP).
4. We detected lower monthly temperature variability in the intermittent reach, likely due to an increased contribution of groundwater to the total runoff, as the contribution of water from upstream was reduced by water diversion.
5. We found an increased air/water temperature slope in the site downstream the WWTP, which led to higher summer temperatures than in adjacent sites.
6. In addition to temperature regime changes, water diversion had remarkable effects on Tordera fish populations. Abundance and survival probability of the two studied fish species, barbel *Barbus meridionalis* and chub *Squalius laietanus*, were lower in the intermittent than in the perennial reaches. Emigration from sampling reaches probably contributed to apparent mortality during extreme flow events.
7. Artificial drought especially affected chub, a species that appears to be declining in recent years. This species is more sensitive than barbel to flow reduction because it is a larger, water-column species that needs deep runs and pools. Barbel seems more adapted than chub to moderate hydrological disturbance, being present at all intermittent reaches (though very scarce after the intense 2012 drought) and showing higher growth rates and somatic condition in the intermittent reaches than in the upstream control reaches. The ability to live in shallow riffles and to escape dryness, and the low competition due to low population density in the intermittent reaches, are probably key aspects of its adaptation.
8. Both fish species were more abundant and showed maximum growth rates and somatic condition in the middle Tordera course, where water flow is permanent

and water quality is poorer due to WWTP effluents, with consequent higher nutrient availability and summer temperatures. Our observations question the usual consideration of *Barbus meridionalis* as an intolerant species, sensitive to water contamination. *Squalius laietanus*, on the other hand, should be considered sensitive to habitat loss.

9. Fish metrics at the population level (abundance and apparent survival) were good indicators of hydrological alteration, in contrast to individual metrics (growth and condition).

REFERENCES

ACA (Agència Catalana de l’Aigua) (2002). Estudi d’actualització de l’avaluació de recursos hídrics de les conques internes de Catalunya i conques catalanes de l’Ebre. Generalitat de Catalunya, Barcelona. Available at http://aca-web.gencat.cat/aca/documents/ca/planificacio/recursos_demandes/recursos_cic.pdf.

Acuña V, Muñoz I, Giorgi A, Omella M, Sabater F, Sabater S (2009). Drought and post-drought recovery cycles in an intermittent Mediterranean stream: structural and functional aspects. Journal of the North American Benthological Society 24: 919–933.

Adams WJ, Kimerle KA, Barnett Jr JW (1992). Sediment quality and aquatic life assessment. Environmental Science and Technology 26: 1865–1875. DOI: 10.1021/es00034a001

Alabaster JS (1970). River flow and upstream movement and catch of migratory salmonids. Journal of Fish Biology 2(1), 1-13.

Alagarsamy R (2006). Distribution and seasonal variation of trace metals in surface sediments of the Mandovi estuary, west coast of India. Estuarine, Coastal and Shelf Science 67: 333–339. DOI: 10.1016/j.ecss.2005.11.023

Albanese B, Angermeier PL, Dorai-Raj S (2004). Ecological correlates of fish movement in a network of Virginia streams. Canadian Journal of Fisheries and Aquatic Sciences 61(6), 857-869.

Alho JM (1990). Logistic regression in capture–recapture models. Biometrics 46:623–635.

Allan JD, Castillo MM (2007). Stream ecology–structure and function of running waters. Springer-Verlag, Dordrecht, The Netherlands.

Almodóvar A, Nicola GG (1999). Effects of a small hydropower station upon brown trout *Salmo trutta* L. in the River Hoz Seca (Tagus Basin, Spain) one year after regulation. Regulated Rivers: Research & Management 15(5), 477-484.

Al-Yousuf MH, El-Shahawi MS, Al-Ghais, SM, (2000). Trace metals in liver, skin and muscle of *Lethrinus lentjan* fish species in relation to body length and sex. Science of the Total Environment 256: 87–94. DOI: 10.1016/S0048-9697(99)00363-0

Andres S, Ribeyre F, Tourencq, J-N, Boudou A (2000). Interspecific comparison of cadmium and zinc contamination in the organs of four fish species along a polymetallic pollution gradient (Lot River, France). Science of the Total Environment 248: 11–25. DOI: 10.1016/S0048-9697(99)00477-5

Angilletta MJ, Ashley Steel E, Bartz KK, Kingsolver JG, Scheuerell MD, Beckman BR, Crozier LG (2008). Big dams and salmon evolution: changes in thermal regimes and their potential evolutionary consequences. Evolutionary Applications 1: 286-299.

Aparicio E, De Sostoa A (1998). Reproduction and growth of *Barbus haasi* in a small stream in the NE of the Iberian peninsula. Archiv für Hydrobiologie,142, 95-110.

Aparicio E, Vargas MJ, Olmo JM, de Sostoa A (2000). Decline of native freshwater fishes in a Mediterranean watershed on the Iberian Peninsula: a quantitative assessment. Environmental Biology of Fishes, 59 (1), 11-19.

Aparicio E, Vargas MJ (2004). Influència de la variabilitat hidrològica sobre les poblacions de peixos de la riera de Fuirosos. In: IV Trobada d'Estudiosos del Montnegre i el Corredor. Diputació de Barcelona, Barcelona, pp. 119–122.

Aparicio E, Vargas MJ, Olmo-Vidal JM (2001). Distribució i característiques poblacionals de la ictiofauna del Parc Natural del Montnegre i el Corredor. In: III Trobada d'Estudiosos del Montnegre i el Corredor. Diputació de Barcelona, Barcelona, pp. 49–53.

Arctic Climate Impact Assessment (ACIA) (2004). Impacts of a warming arctic: arctic climate impact assessment. Cambridge University Press, Oxford, UK

Arscott DB Tockner K, Ward JV (2001). Thermal heterogeneity along a braided floodplain river (Tagliamento River, northeastern Italy). Canadian Journal of Fisheries and Aquatic Sciences 58: 2359-2373.

Arthington AH, Bunn SE, Poff NL, Naiman RJ (2006). The challenge of providing environmental flow rules to sustain river ecosystems. Ecological Applications 16(4), 1311-1318.

Bae M-J, Merciai R, Benejam L, Sabater S, García-Berthou E (2016) Small weirs, big effects: disruption of water temperature regimes with hydrological alteration in a Mediterranean stream. River Research and Applications 32: 309–319.

Barak NA-E, Mason CF (1990). Mercury, cadmium and lead in eels and roach: the effects of size, season and locality on metal concentrations in flesh and liver. Science of the Total Environment 92: 249–256. DOI: 10.1016/0048-9697(90)90334-Q

Bates B, Kundzewicz ZW, Wu S, Palutikof J (2008). Climate change and water: Technical paper vi. Intergovernmental Panel on Climate Change (IPCC).

Bates D, Maechler M, Bolker B, Walker S (2013). Lme4: Linear mixed-effects models using Eigen and s4. R Package version 1.0-5. URL: http://CRAN.R-project.org/package=lme4

Baxter CV, Fausch KD, Carl Saunders W (2005). Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. Freshwater biology 50(2), 201-220.

Beardsley H, Britton JR (2012). Contribution of temperature and nutrient loading to growth rate variation of three cyprinid fishes in a lowland river. Aquatic Ecology 46(1), 143-152.

Benejam L, Aparicio E, Vargas MJ, Vila-Gispert A, García-Berthou E (2008). Assessing fish metrics and biotic indices in a Mediterranean stream: effects of uncertain native status of fish. Hydrobiologia 603: 197-210.

Benejam L, Alcaraz C, Benito J, Caiola N, Casals F, Maceda-Veiga A, de Sostoa A. García-Berthou E. (2012). Fish catchability and comparison of four electrofishing crews in Mediterranean streams. Fisheries Research 123, 9-15.

Benejam L, Angermeier PL, Munné A, García Berthou E (2010). Assessing effects of water abstraction on fish assemblages in Mediterranean streams. Freshwater Biology 55(3), 628-642.

Benejam L, Saura-Mas, S, Bardina M, Solà C, Munné A, García-Berthou, E (2014). Ecological impacts of small hydropower plants on headwater stream fish: from individual to community effects. Ecology of Freshwater Fish 25: 295–306. DOI: 10.1111/eff.12210

Bervoets L, Blust R (2003). Metal concentrations in water, sediment and gudgeon (*Gobio gobio*) from a pollution gradient: relationship with the fish condition factor. Environmental Pollution 126: 9–19. DOI: 10.1016/S0269-7491(03)00173-8

Bestgen KR, Hawkins JA, White GC, Christopherson KD, Hudson JM, Fuller MH, Kitcheyan DC, Brunson R, Badame P, Haines GB, Jackson JA, Walford CD, Sorensen TA (2007). Population status of Colorado pikeminnow in the Green River basin, Utah and Colorado. Transactions of the American Fisheries Society 136: 1356–1380. DOI: 10.1577/T05-303.1

Blanck A, Tedesco PA, Lamouroux N (2007). Relationships between life-history strategies of European freshwater fish species and their habitat preferences. Freshwater Biology 52: 843–859. DOI: 10.1111/j.1365-2427.2007.01736.x

Blinda M, Boufarouna M, Carmi N, Davy T, Detoc S (2007). Technical report on water scarcity and drought management in the Mediterranean and the Water Framework Directive. Mediterranean Water Scarcity & Drought Working Group, European Commission.

Bolin B, Döös BR, Jager J, Warrick RA (1986). The greenhouse effect, climatic change, and ecosystems. John Wiley and Sons, Chichester, UK

Bodine KA, Shoup DE (2010). Capture efficiency of blue catfish electrofishing and the effects of temperature, habitat, and reservoir location on electrofishing-derived length structure indices and relative abundance. North American Journal of Fisheries Management 30 (2), 613-621.

Bohlin T, Hamrin S, Heggberget TG, Rasmussen G, Saltveit SJ (1989). Electrofishing - theory and practice with special emphasis on salmonids. Hydrobiologia 173(1), 9-43.

Bolland JD, Britton JR, Cowx IG (2007). Lifetime consequences of variable 0 year group length in riverine populations of chub *Leuciscus cephalus* (L.). Journal of Fish Biology 71(6), 1810-1819.

Bolton JL, Stehr CM, Boyd DT, Burrows DG, Tkalin AV, Lishavskaya TS, (2004). Organic and trace metal contaminants in sediments and English sole tissues from Vancouver Harbour, Canada. Marine Environmental Research 57: 19-36. DOI: 10.1016/S0141-1136(03)00058-8

Bonada N, Resh VH (2013). Mediterranean-climate streams and rivers: geographically separated but ecologically comparable freshwater systems. Hydrobiologia 719 (1), 1-29.

Bradl H (2005). Heavy metals in the environment: origin, interaction and remediation. 1st ed., Elsevier Academic Press, Amsterdam.

Bramblett RG, Fausch KD (1991) Variable fish communities and the Index of Biotic Integrity in a western Great Plains river. Transactions of the American Fisheries Society, 120, 752–769.

Braune B, Muir D, DeMarch B, Gamberg M, Poole K, Currie R, Dodd M, Duschenko, W, Eamer J, Elkin B, Evans M, Grundy S, Hebert C, Johnstone R, Kidd K, Koenig B, Lockhart L, Marshall H, Reimer K, Sanderson J, Shutt L (1999). Spatial and temporal trends of contaminants in Canadian Arctic freshwater and terrestrial ecosystems: a review, Science of the Total Environment 230: 145–207. DOI: 10.1016/S0048-9697(99)00038-8

Britton J R, Davies GD, Pegg J (2013). Spatial variation in the somatic growth rates of European barbel *Barbus barbus*: a UK perspective. Ecology of Freshwater Fish 22(1), 21-29.

Brown GW, Krygier JT. (1970). Effects of clear-cutting on stream temperature. Water Resources Research 6: 1133-1139.

Brown LE, Hannah DM, Milner AM (2007). Vulnerability of alpine stream biodiversity to shrinking glaciers and snowpacks. Global Change Biology 13: 958-966.

Brown GW (1969). Predicting temperatures of small streams. Water Resources Research 5(1), 68-75.

Brownie C, Hines JE, Nichols JD, Pollock KH, Hestbeck JB (1993). Capture-recapture studies for multiple strata including non-Markovian transitions. Biometrics 49(4), 1173-1187.

Bryan GW, Langston WJ (1992). Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review. Environmental Pollution 76: 89–131. DOI: 10.1016/0269-7491(92)90099-V

Bunn SE, Arthington AH (2002). Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environmental Management 30: 492-507.

Burnham KP, Anderson DR (2003). Model selection and multimodel inference: a practical information-theoretic approach. Springer Science & Business Media.

Caissie D (2006). The thermal regime of rivers: a review. Freshwater Biology 51: 1389–1406.

Candelone JP, Hong S, Pellone C, Boutron CF (1995). Post-Industrial Revolution changes in large-scale atmospheric pollution of the northern hemisphere by heavy metals as documented in central Greenland snow and ice. Journal of Geophysical Research: Atmospheres (1984–2012),100(D8), 16605-16616.

Canli M, Atli G (2003). The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species, Environmental Pollution121: 129–36. DOI: 10.1016/S0269-7491(02)00194-X

Carol J, Benejam L, Alcaraz C, Vila-Gispert A, Zamora L, Navarro E, Armengol J, García-Berthou E (2006). The effects of limnological features on fish assemblages of 14 Spanish reservoirs. Ecology of Freshwater Fish 15: 66–77. DOI: 10.1111/j.1600-0633.2005.00123.x

Carpenter SR, Fisher S, Grimm NB, Kitchell JF (1992). Global change and freshwater ecosystems. Annual Review of Ecology and Systematics, 23, 119-139.

Casals i Martí F, (2005). Les Comunitats íctiques dels rius mediterranis: relació amb les condicions ambientals. Doctoral dissertation, Universitat de Barcelona.

Castleberry DT, Cech JJ (1986). Physiological responses of a native and an introduced desert fish to environmental stressors. Ecology 67(4) 912-918.

Changeux T, Pont D (1995). Current status of the riverine fishes of the French Mediterranean basin. Biological conservation, 72(2), 137-158.

Chugunova NI (1963). Age and growth studies in fish. Israel Program.

Clark RB, (2001). Marine pollution, 5th ed., University Press, Oxford.

Clavero M, Hermoso V, Levin N, Kark S (2010). Biodiversity research: geographical linkages between threats and imperilment in freshwater fish in the Mediterranean Basin. Diversity and Distributions 16(5), 744-754.

Cooper SD, Lake PS, Sabater S, Melack JM, Sabo JL (2013). The effects of land use changes on streams and rivers in mediterranean climates. Hydrobiologia 719(1), 383-425.

Costeja M, Font N, Rigol A, Subirats J (2002). The evolution of the National water regime in Spain. EUWARENESS Project, Universitat Autonoma de Barcelona, Spain.

Crawley MJ (2002). Statistical computing: an introduction to data analysis using S-plus. Chichester: Wiley.

Cudennec C, Leduc C, Koutsoyiannis D (2007). Dryland hydrology in Mediterranean regions - a review. Hydrological Sciences Journal 52(6), 1077-1087.

Custodio E (2012). Low Llobregat Aquifers: Intensive Development, Salinization, Contamination and Management, in: Sabater S, Ginebreda A, Barceló D (Eds.), The Llobregat: The Story of a Polluted Mediterranean River. The Handbook of Environmental Chemistry, Springer, pp. 27–50. DOI: 10.1007/698_2011_138

Dallinger R, Prosi F, Senger H, Back H, (1987). Contaminated food and uptake of heavy metals by fish (a review and proposal for further research). Oecologia 73: 91–98. DOI: 10.1007/BF00376982

Dallman PR, (1998). Plant Life in the World's Mediterranean Climates. California Native Plant Society-University of California Press, Berkeley.

Darwall W, Smith K, Allen D, Seddon M, Reid GM, Clausnitzer V, Kalkman VJ (2009). Freshwater biodiversity: a hidden resource under threat. Wildlife in a Changing World – An Analysis of the 2008 IUCN Red List of Threatened Species, 43.

Davey AJ, Kelly DJ (2007). Fish community responses to drying disturbances in an intermittent stream: a landscape perspective. Freshwater Biology 52(9), 1719-1733.

David BO, Closs GP (2002). Behavior of a stream-dwelling fish before, during, and after high-discharge events. Transactions of the American Fisheries Society 131 (4), 762-771.

Davidson RS, Letcher BH, Nislow KH (2010). Drivers of growth variation in juvenile Atlantic salmon (*Salmo salar*): an elasticity analysis approach. Journal of Animal Ecology 79(5), 1113-1121.

Davies PE, Nelson M (1994). Relationships between riparian buffer widths and the effects of logging on stream habitat, invertebrate community composition and fish abundance. Marine and Freshwater Research 45: 1289-1305.

Dekar MP, Magoulick DD (2013). Effects of predators on fish and crayfish survival in intermittent streams. Southeastern Naturalist 12(1), 197-208.

Demirak A, Yilmaz F, Tuna AL, Ozdemir N (2006). Heavy metals in water, sediment and tissues of *Leuciscus cephalus* from a stream in southwestern Turkey. Chemosphere 63: 1451–8. DOI: 10.1016/j.chemosphere.2005.09.033

Dickson NE, Carrivick JL, Brown LE (2012). Flow regulation alters alpine river thermal regimes. Journal of Hydrology 25: 505–516.

Doadrio I, Kottelat M, de Sostoa A (2007). *Squalius laietanus*, a new species of cyprinid fish from north-eastern Spain and southern France (Teleostei: Cyprinidae). Ichthyological Exploration of Freshwaters 18(3), 247.

Doadrio I, Perea S, Garzón-Heydt P, González JL (2011). Ictiofauna continental española. Bases para su seguimiento. Madrid: Ministerio de Medio Ambiente y Medio Rural y Marino.

Doeg TJ, Koehn JD (1994). Effects of draining and desilting a small weir on downstream fish and macroinvertebrates. Regulated Rivers: Research & Management 9: 263-277.

Dolz J, Puertas J, Herrero E (1994). Water temperature alteration downstream from a reservoir. The Ebro river case (Spain). Commission International de Grands Barrages 69: 211-225.

Dudgeon D (2013). Anthropocene extinctions: global threats to riverine biodiversity and the tragedy of the freshwater commons. River Conservation: Challenges and Opportunities. Sabater S, Elosegi A (eds.). BBVA Foundation, p129-165.

Dynesius M, Nilsson C (1994). Regulation of river systems in the northern third of the world. Science 266(4).

EC, Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union L364 (2006) 0005-0024.

EC, Commission Regulation (EC) No. 629/2008 of 2 July 2008 amending Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union L173 (2008) 0006-0009.

Erickson TR, Stefan HG (2000). Linear air/water temperature correlations for streams during open water periods. Journal of Hydrologic Engineering 5: 317-321.

Fagerstrom T (1977). Body weight, metabolic rate and trace substance turnover in animals. Oecologia 29: 99–104. DOI: 10.1007/BF00345790

Falke JA, Bestgen KR, Fausch KD (2010a). Streamflow reductions and habitat drying affect growth, survival, and recruitment of brassy minnow across a Great Plains riverscape. Transactions of the American Fisheries Society. 139(5), 1566-1583.

Falke JA, Fausch KD, Bestgen KR, Bailey LL (2010b). Spawning phenology and habitat use in a Great Plains, USA, stream fish assemblage: an occupancy estimation approach. Canadian Journal of Fisheries and Aquatic Sciences 67(12), 1942-1956.

Farkas A, Salánki J, Specziár A (2003). Age- and size-specific patterns of heavy metals in the organs of freshwater fish *Abramis brama* L. populating a low-contaminated site. Water Research 37: 959–964. DOI: 10.1016/S0043-1354(02)00447-5

Fernández-Delgado C, Herrera M (1995). Age structure, growth and reproduction of *Leuciscus pyrenaicus* in an intermittent stream in the Guadalquivir river basin, southern Spain. Journal of Fish Biology 46 (3), 371-380.

Ferreira T, Caiola N, Casals F, Oliveira JM, de Sostoa A (2007). Assessing perturbation of river fish communities in the Iberian Ecoregion. Fisheries Management and Ecology, 14, 519–530.

Ferrer R (1994). La gestión integrada de las aguas superficiales y subterraneas del rio Llobregat, Águas Subterráneas. 529-539

Ficke AD, Myrick CA, Hansen LJ (2007). Potential impacts of global climate change on freshwater fisheries. Reviews in Fish Biology and Fisheries17(4), 581-613.

Förstner U, Wittmann GT (2012). Metal pollution in the aquatic environment. Springer Science & Business Media.

Francis R (1990). Back-calculation of fish length: a critical review. Journal of Fish Biology 36(6), 883-902.

García de Jalón D, González del Tánago M, Casado C (1992). Ecology of regulated streams in Spain: an overview. Limnetica 8, 161-166.

García-Berthou E, Moreno-Amich R (1992). Age and growth of an Iberian cyprinodont, *Aphanius iberus* (Cuv. & Val.), in its most northerly population. Journal of Fish Biology, 40(6), 929-937.

García-Berthou E, Moreno-Amich R (1993). Multivariate analysis of covariance in morphometric studies of the reproductive cycle. Canadian Journal of Fisheries and Aquatic Sciences 50(7), 1394-1399.

Garcia-Berthou E, Moreno-Amich R, Coden A (2000). Introduction of exotic fish into a Mediterranean lake over a 90-year period. Archiv für Hydrobiologie 149(2), 271-284.

García-Berthou E, Moreno-Amich R (1993). Multivariate analysis of covariance in morphometric studies of the reproductive cycle, Canadian Journal of Fisheries and Aquatic Sciences 50: 1394–1399. DOI: 10.1139/f93-159

García-Ruiz JM, López-Moreno JI, Vicente-Serrano SM, Lasanta–Martínez T, Beguería S (2011). Mediterranean water resources in a global change scenario. Earth-Science Reviews, 105(3), 121-139.

Gasith A, Resh VH (1999). Streams in Mediterranean Climate Regions: Abiotic Influences and Biotic Responses to Predictable Seasonal Events. Annual Review of Ecology and Systematics 30: 51–81.

Geraldes AM, Collares-Pereira MJ (1995). Preliminary insight into age and growth of the Iberian chub (*Leuciscus pyrenaicus*) in the Sorraia system.

Ginebreda A, Muñoz I, De Alda ML, 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), Environment International 36: 153–162. DOI: 10.1016/j.envint.2009.10.003

Giorgi F (2006). Climate change hot-spots. Geophysical Research Letters 33, L08707, DOI:10.1029/2006GL025734.

González del Tánago M, de Jalón DG, Román M (2012). River restoration in Spain: theoretical and practical approach in the context of the European Water Framework Directive. Environmental Management 50: 123-139.

Gowan C, Fausch KD (1996). Mobile brook trout in two high-elevation Colorado streams: reevaluating the concept of restricted movement. Canadian Journal of Fisheries and Aquatic Sciences 53(6), 1370-1381.

Goyer RA (1997). Toxic and essential metal interactions, Annual Review of Nutrition 17: 37–50.

Goyer RA, Klaassen CD, Waalkes MP (1995). Metal Toxicology, Academic Press, San Diego.

Grabarkiewicz JD, Davis WS (2008). An introduction to freshwater fishes as biological indicators. US Environmental Protection Angecy, Office of Environmental Management.

Grimanis AP, Zafiropoulos D, Vassilaki R, Grimanis M (1978). Trace elements in the flesh and liver of two fish species from polluted and unpolluted areas in the Aegean Sea. Environmental Science and Technology 12: 723–726.

Grimm NB, Faeth SH, Golubiewski NE, Redman CL, Wu J, Bai X, Briggs JM (2008). Global change and the ecology of cities. Science 319: 756–760.

Grossman GD, Ratajczak RE Jr, Cawford M, Freeman MC (1998). Assemblage organization in stream fishes: effects of environmental variation and interspecific interactions. Ecological Monographs 68, 395– 420.

Guasch H, Atli G, Bonet B, Corcoll N, Leira M, Serra A, (2010). Discharge and the response of biofilms to metal exposure in Mediterranean rivers, Hydrobiologia. 657: 143–157. DOI: 10.1007/s10750-010-0116-z

Guy CS, Willis DW (1995). Population characteristics of black crappies in South Dakota waters: a case for ecosystem-specific management. North American Journal of Fisheries Management 15, 754–765.

Hall CA (1972). Migration and metabolism in a temperate stream ecosystem. Ecology, 585-604.

Han FX, Banin A, Su Y, Monts DL, Plodinec JM, Kingery WL, Triplett GE (2002). Industrial age anthropogenic inputs of heavy metals into the pedosphere. Naturwissenschaften 89(11), 497-504.

Harvey BC, Nakamoto RJ, White JL (2006). Reduced streamflow lowers dry-season growth of rainbow trout in a small stream. Transactions of the American Fisheries Society 135(4), 998-1005.

Harvey BC, White JL, Nakamoto RJ, Railsback SF (2014). Effects of streamflow diversion on a fish population: combining empirical data and individual-based models in a site-specific evaluation. North American Journal of Fisheries Management 34(2).

Hermoso V, Clavero M (2011). Threatening processes and conservation management of endemic freshwater fish in the Mediterranean basin: a review. Marine and Freshwater Research 62(3), 244-254.

Herrera M, Fernández-Delgado C (1992). The life-history patterns of *Barbus bocagei sclateri* (Günther, 1868) in a tributary stream of the Guadalquivir River basin, southern Spain. Ecology of Freshwater Fish 1, 42-51.

Herrera M, Fernández-Delgado C (1994). The age, growth and reproduction of Chondrostoma polylepis willkommi in a seasonal stream in the Guadalquivir River basin (southern Spain). Journal of fish Biology 44 (1), 11-22.

Hoenig JM, Morgan MJ, Brown CA (1995). Analysing differences between two age determination methods by tests of symmetry. Canadian Journal of Fisheries and Aquatic Sciences 52(2), 364-368.

Huggins RM (1989). On the statistical analysis of capture experiments. Biometrika 76(1), 133-140.

Huggins RM (1991). Some practical aspects of a conditional likelihood approach to capture experiments. Biometrics 725-732.

Ikem A, Egiebor NO, Nyavor K (2003). Trace elements in water, fish and sediment from Tuskegee Lake, Southeastern USA. Water, Air and Soil Pollution 149: 51–75. DOI: 10.1023/A:1025694315763

Jackson HM, Gibbins CN, Soulsby C (2007). Role of discharge and temperature variation in determining invertebrate community structure in a regulated river. River Research and Applications 23: 651-669.

Johnson FA (1971). Stream temperatures in an Alpine area. Journal of Hydrology 14: 322-336.

Johnson SL (2004). Factors influencing stream temperatures in small streams: substrate effects and a shading experiment. Canadian Journal of Fisheries and Aquatic Sciences 61: 913-923.

Johnson LL, Landahl JT, Kubin LA, Horness BH, Myers MS, Collier TK, Stein JE, (1998). Assessing the effects of anthropogenic stressors on Puget Sound flatfish populations. Journal of Sea Research 39: 125–137. DOI: 10.1016/S1385-1101(97)00057-9

Kalay M, Ay Ö, Canli M, (1999). Heavy metal concentrations in fish tissues from the northeast Mediterrenean Sea. Bulletin of Environmental Contamination and Toxicology 63: 673–681. DOI: 10.1007/s001289901033

Katz RA, Freeman MC (2015). Evidence of population resistance to extreme low flows in a fluvial-dependent fish species. Canadian Journal of Fisheries and Aquatic Sciences 72 (11), 1776-1787.

Keaton M, Haney D, Andersen CB (2005). Impact of drought upon fish assemblage structure in two South Carolina Piedmont streams. Hydrobiologia 545(1), 209-223.

Kendall WL, Nichols JD (1995). On the use of secondary capture-recapture samples to estimate temporary emigration and breeding proportions.Journal of Applied Statistics 22 (5-6), 751-762.

Kendall WL, Nichols JD, Hines JE (1997). Estimating temporary emigration using capture-recapture data with Pollock's robust design. Ecology 78(2), 563-578.

Kinouchi T, Yagi H, Miyamoto M (2007). Increase in stream temperature related to anthropogenic heat input from urban wastewater. Journal of Hydrology 335: 78-88.

Klinger SA, Magnuson JJ, Gallepp GW (1982). Survival mechanisms of the central mudminnow (*Umbra limi*), fathead minnow (*Pimephales promelas*) and brook stickleback (*Culaea inconstans*) for low oxygen in winter. Environmental Biology of Fishes 7(2), 113-120.

Kottelat M, Freyhof J (2007). Handbook of European freshwater fishes, Publications Kottelat, Cornol.

Krebs CJ (1989), Ecological Methodology, New York: Harper Collins.

Kubecka J (1992). Fluctuations in fyke-net catches during the spawning period of the Eurasian perch (*Perca fluviatilis*) in the Rimov Reservoir, Czechoslovakia. Fisheries Research 15(1), 157-167.

Kubečka J, Matěna J, Hartvich P (1997). Adverse ecological effects of small hydropower stations in the Czech Republic: 1. Bypass plants. Regulated rivers: research & management 13(2), 101-113.

Kucuksezgin F, Kontas A, Altay O, Uluturhan E, Darilmaz E (2006). Assessment of marine pollution in Izmir Bay: Nutrient, heavy metal and total hydrocarbon concentrations, Environment International 32: 41-51. DOI: 10.1016/j.envint.2005.04.007

Kuznetsova A, Brockhoff PB, Bojesen-Christensen RH (2013). lmerTest: Tests for random and fixed effects for linear mixed effect models (lmer objects of lme4 package). R Package version 2.0-3. URL: http://CRAN.R-project.org/package=lmerTest

Labbe TR, Fausch KD (2000). Dynamics of intermittent stream habitat regulate persistence of a threatened fish at multiple scales. Ecological Applications 10(6), 1774-1791.

Lagler KF (1966). Freshwater fishery biology.

Lake PS (2003). Ecological effects of perturbation by drought in flowing waters. Freshwater biology 48(7), 1161-1172.

Lappalainen A, Rask M, Koponen H, Vesala S (2001). Relative abundance, diet and growth of perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) at Tvaerminne, northern Baltic Sea, in 1975 and 1997: responses to eutrophication? Boreal Environment Research 6(2), 107-118.

LeBlanc RT, Brown RD, Fitzgibbon JE (1997). Modeling the effects of land use change on the water temperature in unregulated urban streams. Journal of Environmental Management 49: 445-469.

Levin N, Elron E, Gasith, A (2009). Decline of wetland ecosystems in the coastal plain of Israel during the 20th century: implications for wetland conservation and management. Landscape and Urban Planning 92(3), 220-232.

Li HW, Lamberti GA, Pearsons TN, Tait CK, Li JL, Buckhouse JC (1994). Cumulative effects of riparian disturbances along high desert trout streams of the John Day Basin, Oregon. Transactions of the American Fisheries Society 123: 627-640.

Liang Y, Cheung RYH, Wong MH (1999). Reclamation of wastewater for polyculture of freshwater fish: bioaccumulation of trace metals in fish, Water Research 33: 2690–2700. DOI: 10.1016/S0043-1354(98)00473-4

Lobón-Cerviá J, Fernández-Delgado C (1984). On the biology of the barbel (*Barbus barbus bocagei*) in the Jarama river. Folia Zoologica 33(4), 371-384.

Lowney CL (2000). Stream temperature variation in regulated rivers: Evidence for a spatial pattern in daily minimum and maximum magnitudes. Water Resources Research 36: 2947-2955.

Łuczyńska J, Brucka-Jastrzêbska E (2005). The relationship between the content of lead and cadmium in muscle tissue and the size of fish from lakes in the Olsztyn Lake district of northeast Poland, Archives of Polish Fisheries 13: 147–155.

Maceda-Veiga A, De Sostoa A (2011). Observational evidence of the sensitivity of some fish species to environmental stressors in Mediterranean rivers. Ecological Indicators 11(2), 311-317.

Mackey AP, Berrie AD (1991). The prediction of water temperatures in chalk streams from air temperatures. Hydrobiologia 210: 183-189.

MacLeod JC, Pessah, E (1973). Temperature effects on mercury accumulation, toxicity, and metabolic rate in rainbow trout (*Salmo gairdneri*). Journal of the Fisheries Board of Canada 30(4), 485-492.

Magalhães MF (1993). Effects of season and body-size on the distribution and diet of the Iberian chub *Leuciscus pyrenaicus* in a lowland catchment. Journal of Fish Biology 42(6), 875-888.

Magalhães MF, Beja P, Canas C, Collares-Pereira MJ (2002). Functional heterogeneity of dry-season fish refugia across a Mediterranean catchment: the role of habitat and predation. Freshwater Biology 47(10), 1919-1934.

Magalhães MF, Schlosser IJ, Collares-Pereira MJ (2003). The role of life history in the relationship between population dynamics and environmental variability in two Mediterranean stream fishes. Journal of Fish Biology 63(2), 300-317.

Magalhaes MF, Beja P, Schlosser IJ, Collares-Pereira MJ (2007). Effects of multi-year droughts on fish assemblages of seasonally drying Mediterranean streams. Freshwater Biology 52(8), 1494-1510.

Magoulick DD, Kobza RM (2003). The role of refugia for fishes during drought: a review and synthesis. Freshwater biology 48(7), 1186-1198.

Malcolm IA, Soulsby C, Hannah DM, Bacon PJ, Youngson AF, Tetzlaff D (2008). The influence of riparian woodland on stream temperatures: implications for the performance of juvenile salmonids. Hydrological Processes 22: 968-979.

Mann ME, Bradley RS, Hughes MK (1998). Global-scale temperature patterns and climate forcing over the past six centuries. Nature 392:779–787.

Marcé R, Honey-Rosés J, Manzano A, Moragas L, Catllar B, Sabater, S (2012). The Llobregat River Basin: A paradigm of impaired rivers under climate change threats. In The Llobregat (pp. 1-26). Springer Berlin Heidelberg.

Marshall CT, Frank KT (1999). The effect of interannual variation in growth and condition on haddock recruitment. Canadian Journal of Fisheries and Aquatic Sciences 56(3), 347-355.

Mas-Martí E, García-Berthou E, Sabater S, Tomanova S, Muñoz I (2010). Comparing fish assemblages and trophic ecology of permanent and intermittent reaches in a Mediterranean stream. Hydrobiologia 657(1), 167-180.

Matthews WJ (1998). Patterns in Freshwater Fish Ecology. New York: Chapman & Hall.

Matthews WJ, Marsh-Matthews E (2003). Effects of drought on fish across axes of space, time and ecological complexity. Freshwater Biology 48(7), 1232-1253.

McCullagh P, Nelder JA (1989). Generalized linear models. Chapman Hall, London.

McKinley AC, Taylor MD, Johnston EL, (2012). Relationships between body burdens of trace metals (As, Cu, Fe, Hg, Mn, Se, and Zn) and the relative body size of small tooth flounder (*Pseudorhombus jenynsii*), Science of the Total Environment 423: 84–94. DOI: 10.1016/j.scitotenv.2012.02.007

Mendil D, Dogan Uluözlü Ö (2007). Determination of metal levels in sediment of five fish species from lakes in Tokat, Turkey. Food Chemistry 101:739–745. DOI: 10.1016/j.foodchem.2006.01.050

Merseburger GC, Martí E, Sabater F (2005). Net changes in nutrient concentrations below a point source input in two streams draining catchments with contrasting land uses. Science of the Total Environment 347(1), 217-229.

Mildvan AS (1970) Metals in enzyme catalysis. The Enzymes, 3rd Ed. (PD Boyer, ed.) p. 445.

MIMAM (2000). White book of water in Spain. Ministry of the Environment: Madrid; 637 pp. [in Spanish].

Mohseni O, Erickson TR, Stefan HG (1999). Sensitivity of stream temperatures in the United States to air temperatures projected under a global warming scenario. Water Resources Research 35: 3723-3733.

Monikh FA, Safahieh A, Savari A, Doraghi A (2013). Heavy metal concentration in sediment, benthic, benthopelagic, and pelagic fish species from Musa Estuary (Persian Gulf), Environmental Monitoring and Assessment 185: 215–22. DOI: 10.1007/s10661-012-2545-9

Monsefrad F, Imanpour Namin J, Heidary S (2012). Concentration of heavy and toxic metals Cu, Zn , Cd , Pb and Hg in liver and muscles of *Rutilus frisii kutum* during spawning season with respect to growth parameters, Iranian Journal of Fisheries Sciences 11: 825–839.

Munné A, Solà C, Barata C (2012). Human Pressure and Its Effects on Water Quality and Biota in the Llobregat River, in: Sabater S, Ginebreda A, Barceló D (Eds.), The Llobregat: The Story of a Polluted Mediterranean River. Handbook of Environmental Chemistry, Springer, pp. 297-326. DOI: 10.1007/698_2012_149

Muñoz I, García-Berthou E, Sabater S (2012). The effect of multiple stressors on biological communities in the Llobregat, in: Sabater S, Ginebreda A, Barceló D (Eds.), The Llobregat: The Story of a Polluted Mediterranean River, Handbook of Environmental Chemistry, Springer, pp. 93-116. DOI: 10.1007/698_2012_148

Muñoz I, López-Doval JC, Ricart M, Villagrasa M, Brix R, Geiszinger A, Ginebreda A, Guasch H, López de Alda MJ, Romaní AM, Sabater S, Barceló D (2009). Bridging levels of pharmaceuticals in river water with biological community structure in the Llobregat river basin (Northeast Spain), Environmental Toxicology and Chemistry 28: 2706–2714. DOI: 10.1897/08-486.1

Murphy CA, Casals F, Solà C, Caiola N, de Sostoa A, García-Berthou E. (2013). Efficacy of population size structure as a bioassessment tool in freshwaters. Ecological Indicators 34, 571-579.

Murty AS (1986). Toxicity of pesticides to Fish. CRC Press, Boca Raton, FL

Nemcsók J, Orbán L, Aszalos B, Vig E (1987). Accumulation of pesticides in the organs of carp, *Cyprinus carpio* L, at 4° and 20°C. Bulletin of Environmental Contamination and Toxicology 39:370–378

Newman MC, Doubet DK (1989). Size-dependence of mercury (II) accumulation kinetics in the mosquitofish, *Gambusia affinis* (Baird and Girard), Archives of Environmental Contamination and Toxicology 18: 819–25. DOI: 10.1007/BF01160295

Newman MC, Mitz SV (1988). Size dependence of zinc elimination and uptake from water by mosquitofish *Gambusia affinis* (Baird and Girard), Aquatic Toxicology 12: 17-32. DOI: 10.1016/0166-445X(88)90016-1

Nichols JD, Hines JE, Pollock KH (1984). Effects of permanent trap response in capture probability on Jolly-Seber capture-recapture model estimates. The Journal of Wildlife Management, 48(1), 289-294.

Niyogi S, Wood CM (2004). Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals, Environmental Science and Technology 38:6177–6192. DOI: 10.1021/es0496524

Noël L, Chekri R, Millour S, Merlo M, Leblanc J-C, Guérin T (2013). Distribution and relationships of As, Cd, Pb and Hg in freshwater fish from five French fishing areas. Chemosphere 90: 1900–1910. DOI: 10.1016/j.chemosphere.2012.10.015

Nunn AD, Cowx IG, Harvey JP (2002). Recruitment patterns of six species of cyprinid fishes in the lower River Trent, England. Ecology of Freshwater Fish 11, 74–84.

Ogle D (2013). Fisheries stock assessment (fsa) methods package for r. R package version 0.4, 13.

Olden JD, Naiman RJ (2010). Incorporating thermal regimes into environmental flows assessments: modifying dam operations to restore freshwater ecosystem integrity. Freshwater Biology 55: 86-107.

Oliva-Paterna FJ, Miñnano PA, Torralva M (2003a). Habitat quality affects the condition of *Barbus sclateri* in Mediterranean semi-arid streams. Environmental Biology of Fishes 67(1), 13-22.

Oliva-Paterna FJ, Vila-Gispert A, Torralva M (2003b). Condition of *Barbus sclateri* from semi-arid aquatic systems: effects of habitat quality disturbances. Journal of Fish Biology 63(3), 699-709.

Olness A. (1995). Water quality: prevention, identification and management of diffuse pollution. Journal of Environmental Quality 24(2), 383-383.

Ortiz JD, Martí E, Puig M (2006). Influences of a point source on the microhabitat distribution of stream benthic macroinvertebrates. Archiv für Hydrobiologie 165: 469-491.

Ortiz JD, Puig MA (2007). Point source effects on density, biomass and diversity of benthic macroinvertebrates in a Mediterranean stream. River Research and Applications 23: 155-170.

Otero I, Boada M, Badia A, Pla E, Vayreda J, Sabaté S, Gracia CA, Peñuelas J (2011). Loss of water availability and stream biodiversity under land abandonment and climate change in a Mediterranean catchment (Olzinelles, NE Spain). Land Use Policy 28(1), 207-218.

Pankhurst NW, Van Der Kraak G (1997). Effects of stress on reproduction and growth of fish. Fish Stress and Health in Aquaculture, 73-93.

Paquin PR, Gorsuch JW, Apte S, Batley GE, Bowles KC, Campbell PGC, Delos CG, DiToro DM, Dwyer RL, Galvez F, Gensemer RW, Goss GG, Hogstrand C, Janssen, CR, Mcgeer JC, Naddy RB, Playle RC, Santore RC, Schneider U, Stubblefield WA, Wood CM, Wu KB (2002). The biotic ligand model: a historical overview. Comparative Biochemistry and Physiology - Part C 133: 3–35. DOI: 10.1016/S1532-0456(02)00112-6

Patterson KR (1992). An improved method for studying the condition of fish, with an example using Pacific sardine *Sardinops sagax* (Jenyns). Journal of Fish Biology 40, 821–831.

Pérez-Cid B, Boia C, Pombo L, Rebelo E (2001). Determination of trace metals in fish species of the Riade Aveiro (Portugal) by electrothermal atomic absorption spectrometry. Food Chemistry 75: 93–100. DOI: 10.1016/S0308-8146(01)00184-4

Petkovšek SAS, Mazej Grudnik Z, Pokorny B (2012). Heavy metals and arsenic concentrations in ten fish species from the Šalek lakes (Slovenia): assessment of potential human health risk due to fish consumption. Environmental Monitoring and Assessment 184: 2647–2662. DOI: 10.1007/s10661-011-2141-4

Petrovic M, Barceló D (2012). Inputs of pharmaceuticals and endocrine disrupting compounds in the Llobregat River basin, in: Sabater S, Ginebreda A, Barceló D (Eds.), The Llobregat: The Story of a Polluted Mediterranean River. Handbook of Environmental Chemistry, Springer, pp. 151-166. DOI: 10.1007/698_2011_141

Pires AM, Cowx IG, Coelho MM (1999). Seasonal changes in fish community structure of intermittent streams in the middle reaches of the Guadiana basin, Portugal. Journal of Fish Biology 54(2), 235-249.

Pires DF, Beja P, Magalhães MF (2014). Out of pools: movement patterns of Mediterranean stream fish in relation to dry season refugia. River Research and Applications 30(10), 1269-1280.

Pires DF (2012). Fish distribution and abundance in mediterranean streams:the role of habitat quality, spatial context, and movement patterns. PhD thesis, Faculdade de Ciências da Universidade de Lisboa: Lisboa.

Poff NL, Zimmerman JK (2010). Ecological responses to altered flow regimes: a literature review to inform the science and management of environmental flows. Freshwater Biology 55(1), 194-205.

Poff NL, Olden JD, Merritt DM, Pepin DM (2007). Homogenization of regional river dynamics by dams and global biodiversity implications. Proceedings of the National Academy of Sciences 104(14), 5732-5737.

Pollock KH (1982). A capture-recapture design robust to unequal probability of capture. The Journal of Wildlife Management 46(3), 752-757.

Pollock KH, Nichols JD, Brownie C, Hines JE (1990). Statistical inference for capture-recapture experiments. Wildlife Monographs 3-97.

Poole GC, Berman CH (2001). An ecological perspective on in-stream temperature: natural heat dynamics and mechanisms of human-caused thermal degradation. Environmental Management 27: 787–802.

Prat N, Munné A, Solà C, Casanovas-Berenguer R, Vila-Escalé M, Bonada N, Jubany J, Miralles M, Plans M. & Rieradevall M (2002) La qualitat ecològica del Llobregat, el Besòs, el Foix i la Tordera. Diputació de Barcelona, Barcelona.

Prat N, Rieradevall M (2006). 25-years of biomonitoring in two mediterranean streams (Llobregat and Besòs basins , NE Spain). Limnetica 25: 541–550.

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

Preece R (2004). Cold water pollution below dams in New South Wales: a desktop assessment. NSW Department of Infrastructure, Planning and Natural Resources, Sydney.

R Development Core Team (2011) – FSA package

Rajkowska M, Protasowicki M (2013). Distribution of metals (Fe, Mn, Zn, Cu) in fish tissues in two lakes of different trophy in Northwestern Poland. Environtal Monitoring and Assessment 185: 3493–3502. DOI: 10.1007/s10661-012-2805-8

Rejomon G, Nair M, Joseph T (2010). Trace metal dynamics in fishes from the south west coast of India. Environtal Monitoring and Assessment 167: 243–255. DOI: 10.1007/s10661-009-1046-y

Reyjol Y, Hugueny B, Pont D, Bianco PG, Beier U, Caiola N, Casals F, Cowx I, Economou A, Ferreira T, Haidvolg G, Noble R, de Sostoa A, Vigneron T, Virbickas T (2007). Patterns in species richness and endemism of European freshwater fish. Global Ecology and Biogeography 16, 65–75.

Reynolds JB, (1983). Electrofishing. In: Nielsen LA, Johnson DL (Eds.), Fisheries Techniques. American Fisheries Society, Bethesda, MD, pp. 147–163.

Ricart M, Guasch H, Barceló D, Brix R, Conceição MH, Geiszinger A, López de Alda, MJ, López-Doval JC, Muñoz I, Postigo C, Romaní AM, Villagrasa M, Sabater S (2010). Primary and complex stressors in polluted mediterranean rivers: Pesticide effects on biological communities. Journal of Hydrology 383: 52–61. DOI: 10.1016/j.jhydrol.2009.08.014

Riley SC, Fausch, KD (1992). Underestimation of trout population size by maximum-likelihood removal estimates in small streams. North American Journal of Fisheries Management 12(4), 768-776.

Roberts JH, Rosenberger AE, Albanese BW, Angermeier PL (2008). Movement patterns of endangered Roanoke logperch (*Percina rex*). Ecology of Freshwater Fish 17: 374–381.

Rovira A, Batalla RJ (2006). Temporal distribution of suspended sediment transport in a Mediterranean basin: The Lower Tordera (NE SPAIN). Geomorphology 79(1), 58-71.

Rozon-Ramilo LD, Dubé MG, Squires AJ, Niyogi S (2011). Examining waterborne and dietborne routes of exposure and their contribution to biological response patterns in fathead minnow (*Pimephales promelas*), Aquatic Toxicology 105: 466–481. DOI: 10.1016/j.aquatox.2011.07.006

Sabater S (2008). Alterations of the global water cycle and their effects on river structure, function and services. Freshwater Reviews 1: 75-88.

Sabater S, Tockner K (2010). Effects of hydrologic alterations on the ecological quality of river ecosystems. In: Water scarcity in the Mediterranean (pp. 15-39). Springer Berlin Heidelberg.

Sabater S, Guasch H, Kumar A, Osorio V, Perez S, Ponsatí L, Ricart M, Petrovic M, Barceló D (2012). Pollution in Mediterranean river ecosystems of the Iberian Peninsula: effects on benthic communities, in: 1ère conférence internationale sur les recherches et actions au service des fleuves et grandes rivières, 26-28 June, Lyon, France: pp. 1–3.

Schlosser IJ (1990). Environmental variation, life history attributes, and community structure in stream fishes: implications for environmental management and assessment. Environmental Management, 14(5), 621-628.

Schlosser, I. J. 1995. Dispersal, boundary processes, and trophic-level interactions in streams adjacent to beaver ponds. Ecology 76: 908–925. DOI: 10.2307/1939356

Serra R, Carpene E, Torresani G, Andreucci A, Grandini S (1993). Concentrations of Zn, Cu, Fe, and Cd in *Liza ramada* and *Leuciscus cephalus*, Archivio Veterinario Italiano 44: 166–174.

Servat E (2003). Hydrology of Mediterranean and Semiarid Regions: Papers Selected for the International Conference on Hydrology of the Mediterranean and Semi-Arid Regions, Held in Montpellier, France from 1 to 4 April 2003 (No. 278). International Association of Hydrological Sciences.

Shinn C, Dauba F, Grenouillet G, Guenard G, Lek S, (2009). Temporal variation of heavy metal contamination in fish of the river Lot in southern France, Ecotoxicology and Environmental Safety 72: 1957–1965. DOI: 10.1016/j.ecoenv.2009.06.007

Shul'man GE (1974). Life cycles of fish, Wiley, New York.

Sinokrot BA, Gulliver JS. (2000). In-stream flow impact on river water temperatures. Journal of Hydraulic Research 38: 339-349.

Sinokrot BA, Stefan HG, McCormick JH, Eaton JG (1995). Modeling of climate change effects on stream temperatures and fish habitat below dams and enar groundwater inputs. Climatic Change 30: 180-200.

Smith K, Lavis ME (1975). Environmental influences on the temperature of a small upland stream. Oikos 26: 228–236.

Smith KG, Darwall WR (Eds.) (2006). The status and distribution of freshwater fish endemic to the Mediterranean Basin (Vol. 1). IUCN.

Smith KG and Darwall WR (Compilers) (2006). The Status and Distribution of Freshwater Fish Endemic to the Mediterranean Basin. IUCN, Gland, Switzerland and Cambridge, UK. v + 34 pp.

Speas DW, Walters CJ, Ward DL, Rogers RS (2004). Effects of intraspecific density and environmental variables on electrofishing catchability of brown and rainbow trout in the Colorado River. North American Journal of Fisheries Management 24(2), 586-596.

Spranza JJ, Stanley EH (2000). Condition, growth, and reproductive styles of fishes exposed to different environmental regimes in a prairie drainage. Environmental Biology of Fishes 59(1), 99-109.

Spray SL, McGlothlin KL (2002) Global climate change. Rowman and Littlefield, New York

Steel EA, Lange IA (2007). Using wavelet analysis to detect changes in water temperature regimes at multiple scales: effects of multi-purpose dams in the Willamette River basin. River Research and Applications 23: 351-359.

Stevenson RJ, Sabater S (2010). Understanding effects of global change on river ecosystems: science to support policy in a changing world. In: Global Change and River Ecosystems - Implications for Structure, Function and Ecosystem Services (pp. 3-18). Springer Netherlands.

Taylor MK, Cooke SJ (2012). Meta-analyses of the effects of river flow on fish movement and activity. Environmental Reviews 20 (4), 211-219.

Teichert MAK, Kvingedal E, Forseth T, Ugedal O, Finstad AG (2010). Effects of discharge and local density on the growth of juvenile Atlantic salmon *Salmo salar*. Journal of Fish Biology 76(7), 1751-1769.

Terra BF, Araújo FG, Calza CF, Lopes RT, Teixeira TP (2008). Heavy metal in tissues of three fish species from different trophic levels in a tropical Brazilian river. Water, Air and Soil Pollution 187: 275–284. DOI: 10.1007/s11270-007-9515-9

Todd CR, Ryan T, Nicol SJ, Bearlin AR (2005). The impact of cold water releases on the critical period of post-spawning survival and its implications for Murray cod (*Maccullochella peelii peelii*): a case study of the Mitta Mitta River, southeastern Australia. River Research and Applications 21: 1035-1052.

Vetter EF (1988). Estimation of natural mortality in fish stocks: a review. Fishery Bulletin 86(1), 25-43.

Vila-Gispert A, Moreno-Amich R (2001). Mass-length relationship of Mediterranean barbel as an indicator of environmental status in South-west European stream ecosystems. Journal of Fish Biology 59(4), 824-832.

Vila-Gispert A, Zamora L, Moreno-Amich R (2000). Use of the condition of Mediterranean barbel (*Barbus meridionalis*) to assess habitat quality in stream ecosystems. Archiv für Hydrobiologie 148(1), 135-145.

Ward JV (1985). Thermal characteristics of running waters. Hydrobiologia 125: 31-46.

Weatherly AH, Gill HS (1987). The biology of fish growth, Academic Press, Orlando, FL.

Webb BW (1992). Climate change and the thermal regime of rivers. Rep. for the Department of the Environment under Contract PECD/7/7/348,University of Exeter, Department of Geography, UK.

Webb BW, Walling DE (1993). Temporal variability in the impact of river regulation on thermal regime and some biological implications. Freshwater Biology 29: 167-182.

Webb BW, Walling DE (1996). Long-term variability in the thermal impact of river impoundment and regulation. Applied Geography 16: 211-223.

Webb BW (1996). Trends in stream and river temperature. Hydrological Processes 10: 205–226.

Weisberg S (2012). Mixed effects models for fish growth. URL: http://www. stat.umn.edu/~sandy/courses/8053/handouts/fishgrowth.pdf

Weisberg S, Spangler G, Richmond LS (2010). Mixed effects models for fish growth. Canadian Journal of Fisheries and Aquatic Sciences 67(2), 269-277.

White GC, Burnham KP (1999). Program MARK: survival estimation from populations of marked animals. Bird Study 46(S1), S120-S139.

Williams DR, Giesy Jr JP (1978). Relative importance of food and water sources to cadmium uptake by *Gambusia affinis* (Poeciliidae), Environmental Research 16: 326–332. DOI: 10.1016/0013-9351(78)90165-2

Winemiller KO, Rose KA (1992). Patterns of life-history diversification in North American fishes: implications for population regulation. Canadian Journal of Fisheries and Aquatic Sciences 49(10), 2196-2218.

Xenopoulos MA, Lodge DM, Alcamo J, Märker M, Schulze K, Van Vuuren DP (2005). Scenarios of freshwater fish extinctions from climate change and water withdrawal. Global Change Biology 11(10), 1557-1564.

Xin Z, Kinouchi T (2013). Analysis of stream temperature and heat budget in an urban river under strong anthropogenic influences. Journal of Hydrology 489: 16-25.

Yi Y-J, Zhang S-H, (2012). Heavy metal (Cd, Cr, Cu, Hg, Pb, Zn) concentrations in seven fish species in relation to fish size and location along the Yangtze River. Environmental Science and Pollution Research 19: 3989–3996. DOI: 10.1007/s11356-012-0840-1

Zelasko KA, Bestgen KR, White GC (2010). Survival rates and movement of hatchery-reared razorback suckers in the upper Colorado River basin, Utah and Colorado. Transactions of the American Fisheries Society 139(5), 1478-1499.

Zoumis T, Schmidt A, Grigorova L, Calmano W (2001). Contaminants in sediments: remobilization and demobilisation, Science of the Total Environment 266: 195–202. DOI: 10.1016/S0048-9697(00)00740-3

Winters GH, Wheeler JP (1994). Length-specific weight as a measure of growth success of adult Atlantic herring (*Clupea harengus*). Canadian Journal of Fisheries and Aquatic Sciences 51, 1169–1179.