CONSERVATION ECOLOGY OF HEADWATER STREAMS:
A CASE STUDY FROM THE PYRENEES

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DOCTORAL THESIS

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Doctoral Programme in Water Science and Technology

Supervised by:

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

Dr. Helena Guasch Padró from the Continental Ecology Department of CEAB-CSIC, and Dr. Anna Vila Gispert from the Institute of Aquatic Ecology of the University of Girona,

DECLARE:

That the thesis entitled “**Conservation ecology of headwater streams: a case study from the Pyrenees**” presented by **ALEJANDRO LÓPEZ DE SANCHA** to obtain a Doctoral degree, has been conducted under our supervision.

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

Dr. Helena Guasch Padró		Dr. Anna Vila Gispert

Girona, May 2022

“*Acts of goodness are not always wise, and acts of evil are not always foolish, but regardless, we shall always strive to be good.*”

– Hidetaka Miyazaki

Agraïments

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List of publications

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- **López-de Sancha, A.**, Roig, R., Vila-Gispert, A. and Guasch, H. (2022). River trout density role on the ecosystemic structure of a Pyrenean headwater stream. Limnetica, *In press.*

List of abbreviations

ACA: Agència Catalana de l'Aigua

AFDM: Ash Free Dry Mass

A.I.: Autotrophic Index

ANCOVA: Analysis of Covariance

ANOVA: Analysis of Variance

As: Arsenic

Bd: *Batrachochytrium dendrobatidis*

Bsal: *Bathracochytrium salamandrivorans*

Cd: Cadmium

Chl-*a*: Chlorophyll-*a*

CPOM: Coarse Particulate Organic Matter

Cr: Chromium

Cu: Copper

Dim: Dimension

DL: Detection Limit

Down: Downstream

EPS: Extracellular Polymeric Substances

EPT: Ephemeroptera, Plecoptera and Trichoptera Index

FFG: Functional Feeding Group

FHI: Fluvial Habitat Index

FPOM: Fine Particulate Organic Matter

IASPT: Iberian Average Score per Taxon

IBMWP: Iberian Biological Monitoring Working Party

ICP-MS: Inductively Coupled Plasma Mass Spectrometry

Ind: Individuals

LME: Linear Mixed-Effects model

m.a.s.l.: meters above sea level

MW: Mega Watts

NE: North-East

Ni: Nickel

NMDS: Non-metric Multidimensional Scaling

PCA: Principal Components Analysis

PCR: Polymerase Chain Reaction

P.R.: Preciptiation Regime

Sb: Antimony

SHP: Small Hydropower Plant

SVL: Snout-to-vent length

TL: Total Length

Upst: Upstream

Zn: Zinc

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GENERAL DISCUSSION

Resum

Les rieres de muntanya del Pirineu són ecosistemes singulars, amb característiques abiòtiques particulars que segueixen un patró estacional. Sustenten una biodiversitat única i especialment sensible als canvis ambientals i, degut a això, la seva estructura i funció ecosistèmica està amenaçada per múltiples factors d'estrès tals com la reducció en les precipitacions promoguda pel canvi climàtic, la presència de preses, la contaminació per metalls derivada de la mineria i altres activitats humanes. Una anàlisis integrativa d'aquests impactes utilitzant múltiples bioindicadors a la vegada, amb un enfoc tant estructural com funcional, pot omplir l'actual buit de coneixement sobre l'avaluació de la vulnerabilitat de les rieres de muntanya davant d'aquests factors d'estrès. Aquestes rieres són fonamentals per a la integritat ecològica de les conques hidrològiques, però encara manca el coneixement necessari per la proposta d'actuacions de gestió adients. Els Pirineus en són un exemple ja que estan greument amenaçats pel canvi climàtic, i moltes de les seves rieres estan afectades per la contaminació per metalls i per la presència de preses. A més, s'espera un increment en la demanda d'aigua a aquesta regió al llarg d'aquest segle, el que augmenta la incertesa sobre la suficient disponibilitat d'aigua i fa necessària l'adopció de noves mesures de gestió. En aquest context, és necessari un enfoc de recerca aplicada per tal de gestionar i conservar efectivament aquests ecosistemes tan amenaçats.

En base a aquestes necessitats, aquesta tesi ha escollit com a cas d'estudi una riera de muntanya dels Pirineus on hi ha una presa hidroelèctrica i que rep l'escorrentia d'afluents que drenen d'unes antigues mines d'antimoni. En aquest cas d'estudi s'ha avaluat **i)** com l'estacionalitat, la presència de la presa i dels diferents règims de precipitació modulen les condicions abiòtiques i, en conseqüència, les comunitats del biofilm i dels macroinvertebrats d'aquests ecosistemes, **ii)** com la comunitat de macroinvertebrats actua com a bioindicador d'impactes antropogènics que afecten l'estructura i funció d'aquests ecosistemes, **iii)** l'ús dels macroinvertebrats com a recurs tròfic per a depredadors vertebrats d'aquestes rieres en el context canviant actual, i **iv)** els impactes de la presa i de la contaminació per metalls sobre el tritó pirinenc (*Calotriton asper*).

Al ***Capítol 1***, es va determinar al llarg de dos anys, mitjançant l'estudi de les comunitats del biofilm i dels macroinvertebrats d'una riera de muntanya Pirenaica, la resposta de variables relacionades amb l'estructura de les comunitats biològiques davant de l'estacionalitat, la presència d'una presa,

diferents règims de precipitació i davant de la contaminació per metalls. Les mètriques analitzades seguien un patró estacional que es veia alterat per la presa i per una reducció en les precipitacions, fets que implicaven una reducció de cabal i un augment de la temperatura de l'aigua. Aquesta situació promovia un increment de biomassa algal i de macroinvertebrats, a expenses de la seva biodiversitat, una situació que s'espera que empitjori dins de l'actual context de canvi climàtic. La reducció de cabal durant el règim de precipitació sec incrementava la concentració de metalls i metal·loides de la riera, però la seva concentració sota la presa disminuïa donada la diversió d'aigua de la presa per un canal adjacent. Aquests resultats suggereixen que s'ha de mantenir un cabal ecològic suficient a les rieres afectades per preses si es pretén superar els impactes abans descrits dins d'aquest context climàtic. A més, demostren la necessitat d'estudis de camp per a determinar com múltiples factors d'estrès antropogènics interactuen i amenacen la integritat ecològica de les rieres de muntanya.

Al ***Capítol 2***, es va utilitzar la comunitat de macroinvertebrats de la riera com a bioindicador de l'alteració de funcions ecosistèmiques de la riera. Es van avaluar els efectes de l'estacionalitat, de la presència d'una presa i dels diferents règims de precipitació sobre diferents funcions ecosistèmiques calculades de manera indirecta a partir de dades taxonòmiques de la comunitat de macroinvertebrats. La reducció de cabal provocada per la presa i la disminució en les precipitacions promovia l'autotròfia de la riera, alterant el patró estacional natural de la comunitat de macroinvertebrats, disminuint la seva diversitat i afectant a la proporció de diferents grups tròfics. Aquest canvi estructural en la comunitat es traduïa en impactes en la funcionalitat de l'ecosistema, ja que es va alterar la direcció de la xarxa tròfica. La intensificació de processos tròfics *bottom-up* lligats a aquesta promoció de l'autotròfia reduïa els processos *top-down*, disminuint així l'abundància de macroinvertebrats depredadors.

Al ***Capítol 3***, es va analitzar el solapament de la dieta entre tres espècies insectívores de vertebrats, emblemàtiques de les rieres del Pirineu: *Galemys pyrenaicus*, *Neomys fodiens* i *Cinclus cinclus*. Aquest solapament de dieta implicava una competència tròfica en la qual l'almesquera (*G. pyrenaicus*) s'especialitzava en preses aquàtiques energètiques i altament disponibles a les aigües ràpides de la riera, degut a les seves adaptacions fisiològiques i morfològiques que li permeten capturar preses aquàtiques de forma eficient, mentre evitava les preses terrestres. Això fa que aquesta espècie sigui altament vulnerable a una disminució en la disponibilitat de recursos alimentaris i als factors d'estrès antropogènics que fan disminuir la qualitat de l'hàbitat. Els resultats obtinguts mostren la importància de l'hàbitat, indicant la necessitat de que la riera mantingui nombroses seccions d'aigües ràpides per tal de proveir suficient hàbitat de qualitat i preses per a l'almesquera que li permetin utilitzar les seves adaptacions de manera efectiva. Es considera que la reducció de cabal i la pèrdua de connectivitat que causen les preses representen una amenaça seriosa per a la conservació d'aquesta espècie. En aquest estudi també es conclou que les anàlisis de dieta són efectives per a avaluar els requeriments alimentaris d'espècies amenaçades com l'almesquera, però que han de considerar l'ecologia de les preses de les que s'alimenta per tal d'evitar errors als anàlisis d'electivitat de preses.

Al ***Capítol 4***, es va descriure una nova població de l'amenaçat tritó pirinenc *Calotriton asper*, i es van avaluar els impactes de la presa i de la contaminació per metalls en ella. Es va comparar la densitat poblacional, la mida dels individus i la prevalença de la quitridiomicosi a ambdues bandes de la presa. Utilitzant dades prèvies de la tesi, es va determinar que la contaminació per metalls causava una disminució tant de la densitat poblacional com de la mida dels individus a la subpoblació situada sobre la presa. D'altra banda, no es van trobar individus afectats per quitridiomicosi a la riera, tot i trobar-se a només 8 Km d'una població infectada. Es necessita més recerca per a determinar els impactes dels metalls i metal·loides i les preses sobre el tritó pirinenc per tal de gestionar petites poblacions d'aquesta espècie a rieres afectades per aquestes amenaces i millorar així la seva conservació.

Els resultats obtinguts en aquesta tesi aporten, dins d'un context de conservació, nova informació sobre els efectes de la interacció entre els impactes del canvi climàtic, la presència de preses i la contaminació per metalls sobre l'estructura i funció de les comunitats d'organismes aquàtics que viuen en rieres de muntanya. Es va observar que una reducció de les precipitacions tenia un impacte sinèrgic amb la presa al reduir el cabal d'aigua de la riera, el que promovia l'autotròfia de la riera i reduïa la seva biodiversitat, alterant també la seva funcionalitat. Aquests resultats aporten evidències científiques útils per a la proposta de mesures efectives per la conservació i gestió d'aquests ecosistemes, tals com el manteniment d'un cabal ecològic, especialment si es considera que cada riera pot estar afectada per amenaces addicionals com pot ser la contaminació per metalls. Les conseqüències d'aquestes amenaces són especialment rellevants per a espècies clau d'aquestes rieres. D'una banda, la disponibilitat de recursos basals pot veure's amenaçada, incrementant la competència per determinades preses per part de nivells tròfics superiors, desplaçant així a espècies com l'almesquera. D'altra banda, la interacció de la presa amb la contaminació per metalls pot

afectar la salut i la distribució de poblacions d'espècies sensibles com el tritó pirinenc. Aquesta tesi il·lustra que un enfoc integratiu, incloent les relacions tròfiques, és útil per a entendre els impactes antropogènics en la biodiversitat de les rieres de muntanya i per a proposar actuacions efectives de gestió i conservació.

Resumen

Los arroyos de montaña del Pirineo son ecosistemas singulares, con características abióticas particulares que siguen un patrón estacional. Sustentan una biodiversidad única y especialmente sensible a los cambios ambientales y, debido a esto, su estructura y función ecosistémica está amenazada por múltiples factores de estrés, como una reducción en las precipitaciones promovida por el cambio climático, la presencia de presas, y la contaminación por metales derivada de la minería y otras actividades humanas. Un análisis integrativo de estos impactos utilizando múltiples bioindicadores a la vez, con un enfoque tanto estructural como funcional, puede llenar el actual vacío de conocimiento sobre la evaluación de la vulnerabilidad de los arroyos de montaña frente a estos factores de estrés. Estos arroyos son fundamentales para la integridad ecológica de cuencas hidrográficas, pero aún falta el conocimiento necesario para proponer actuaciones de gestión pertinentes. Los Pirineos son un ejemplo de ello, ya que están gravemente amenazados por el cambio climático y muchos de sus arroyos están afectados por metales y presas. Además, se espera un incremento de la demanda de agua en esta región a lo largo de este siglo, lo que incrementa la incertidumbre de una suficiente disponibilidad de agua a lo largo de este siglo y hace necesaria la adopción de nuevas medidas de gestión. En este contexto, es necesario un enfoque de investigación aplicada por tal de gestionar y conservar efectivamente estos ecosistemas tan amenazados.

En base a estas necesidades, esta tesis ha elegido como caso de estudio un arroyo de montaña de los Pirineos donde se encuentra una presa hidroeléctrica y que recibe la escorrentía de afluentes que drenan de unas antiguas minas de antimonio. En este caso de estudio se ha evaluado **i)** cómo la estacionalidad, la presencia de la presa y diferentes regímenes de precipitación modulan las condiciones abióticas y, en consecuencia, las comunidades de biofilms y macroinvertebrados de estos ecosistemas, **ii)** cómo la comunidad de macroinvertebrados actúa como bioindicador de impactos antropogénicos sobre la estructura y función de estos ecosistemas, **iii)** el uso

de los macroinvertebrados como recurso trófico por parte de depredadores vertebrados en estos arroyos en el contexto de cambio actual, y **iv)** los impactos de la presa y la contaminación por metales sobre el tritón pirenaico (*Calotriton asper*).

En el ***Capítulo 1***, se determinó a lo largo de dos años, mediante el estudio de las comunidades del biofilm y de los macroinvertebrados de un arroyo Pirenaico, la respuesta de las variables relacionadas con la estructura ecosistémica de estas comunidades frente a la a estacionalidad, la presencia de una presa, diferentes regímenes de precipitación, y frente a la contaminación por metales. Las métricas analizadas seguían un patrón estacional que era alterado por la presa y por una reducción en las precipitaciones, ya que implicaban una reducción del caudal y un aumento de la temperatura del agua, promoviendo un incremento de la biomasa algal y de macroinvertebrados a costa de su biodiversidad, una situación que se espera que empeore dentro del contexto actual de cambio climático. La reducción de caudal durante el régimen de precipitación seco incrementaba la concentración de metales y metaloides del arroyo, pero su concentración bajo la presa disminuía dada la diversión de agua por un canal adyacente. Estos resultados sugieren que se debe mantener un caudal ecológico suficiente en los arroyos afectados por presas en este contexto climático. Además, demuestran la necesidad de estudios de campo para determinar cómo múltiples factores de estrés antropogénicos interactúan y amenazan la integridad ecológica de los arroyos de montaña.

En el ***Capítulo 2***, se analizó la utilidad de la comunidad de macroinvertebrados del arroyo como un bioindicador de la alteración de las funciones ecosistémicas. Se evaluaron los efectos de la estacionalidad, la presencia de una presa, y de los diferentes regímenes de precipitación sobre diferentes funciones ecosistémicas calculadas de manera indirecta a partir de datos taxonómicos de la comunidad de macroinvertebrados. La reducción de caudal causada por la presa y la disminución en las precipitaciones promovía la autotrofia del arroyo, alterando el patrón estacional natural que estructura el ensamblaje de la comunidad de macroinvertebrados, disminuyendo su diversidad y afectando la proporción de diferentes grupos tróficos. Estos cambios estructurales se traducían en impactos en la funcionalidad del ecosistema, ya que se alteró la dirección de la red trófica. La intensificación de procesos tróficos *bottom-up* vinculada a esta promoción de la autotrofia reducía los procesos *top-down*, disminuyendo así la abundancia de macroinvertebrados depredadores.

En el ***Capítulo 3***, se analizó el solapamiento de dieta entre tres especies insectívoras de vertebrados, emblemáticos de los arroyos del Pirineo: *Galemys pyrenaicus, Neomys fodiens* y *Cinclus cinclus*. Este solapamiento implicaba una competencia trófica en la que el desmán *G. pyrenaicus* se especializaba en presas acuáticas energéticas y altamente disponibles en las aguas rápidas del arroyo, debido a sus adaptaciones morfológicas y fisiológicas que le permiten capturar esas presas acuáticas de forma eficiente, mientras evita las terrestres. Esto hace esta especie en altamente vulnerable a una disminución de la disponibilidad de recursos alimentarios y a los factores de estrés antropogénicos que hacen disminuir la calidad del hábitat. Los resultados obtenidos muestran la importancia del hábitat, indicando la necesidad de que el arroyo mantenga numerosas secciones de aguas rápidas para proveer de suficiente hábitat de calidad y presas para el desmán, permitiéndole utilizar sus adaptaciones de manera efectiva. Se considera que una reducción del caudal y la pérdida de conectividad que las presas causan es una amenaza seria para la conservación de esta especie. En este estudio también se concluyó que los análisis de dieta son efectivos para evaluar los requerimientos alimentarios de especies amenazadas como el desmán, pero que deben considerar la ecología de las presas de las que se alimenta para evitar sesgos en los análisis de electividad de presas.

En el ***Capítulo 4***, se describió una nueva población del amenazado tritón pirenaico *Calotriton asper*, y se evaluaron los impactos de la presa y la contaminación por metales en ella. Se comparó la densidad poblacional, el tamaño de los individuos y la prevalencia de la quitridiomicosi en ambas bandas de la presa. Se determinó que la contaminación por metales causaba una disminución tanto de la densidad poblacional como del tamaño de los individuos en la subpoblación de arriba de la presa. Por otro lado, no se encontraron individuos afectados por quitridiomicosi en el arroyo a pesar de encontrarse este tan solo a 8 Km de una población infectada. Se necesita más investigación para determinar los impactos de los metales y los metaloides y las presas sobre el tritón pirenaico con tal de gestionar pequeñas poblaciones de esta especie en arroyos afectados por estas amenazas y mejorar así su conservación.

Los resultados de esta tesis aportan, en un contexto de conservación, nueva información sobre los efectos de la interacción entre los impactos del cambio climático, la presencia de presas y la contaminación por metales sobre la estructura y función de las comunidades de organismos acuáticos de los arroyos de montaña. Se observó que una reducción de las precipitaciones tenía un impacto sinérgico con la presa al reducir el caudal del arroyo,

promoviendo su autotrofia y reduciendo su biodiversidad, alterando también su funcionalidad. Estos resultados aportan evidencias científicas para la propuesta de medidas efectivas para la conservación y gestión de esos ecosistemas, como el mantenimiento de un caudal ecológico, especialmente considerando que cada arroyo puede estar afectado por amenazas adicionales como puede ser la contaminación por metales. Las consecuencias de estas amenazas son especialmente relevantes para especies clave en estos arroyos. De una banda, la disponibilidad de recursos basales puede verse amenazada, incrementando la competencia por determinadas presas en niveles tróficos superiores, desplazando así a especies como el desmán. De otra banda, la interacción de la presa con la contaminación por metales puede afectar la salud y distribución de poblaciones de especies sensibles como el tritón pirenaico. Esta tesis ilustra que un enfoque integrativo, incluyendo relaciones tróficas, es útil para entender los impactos antropogénicos en la biodiversidad de los arroyos de montaña y para proponer medias efectivas de gestión y conservación.

Summary

Pyrenean headwater streams are singular ecosystems, with particular and sensitive abiotic characteristics in a seasonal pattern, that sustain a unique biodiversity especially sensitive to environmental change. Because of this, their ecosystem structure and function are highly threatened by many anthropogenic stressors such as the precipitation decrease and water warming promoted by climate change, damming, and metal pollution derived from mining and other human activities. An integrative assessment of these impacts by using multiple bioindicators at the same time, with both a structural and functional approach, can fill the current knowledge gap for the correct evaluation of the vulnerability of headwater streams to those stressors. This kind of streams are fundamental for the ecological integrity of whole river networks, but there is still a need of more knowledge to propose adequate management actions. Pyrenean headwater streams are a great example to appraise these topics as they are threatened by climate change and many of them are affected by damming and metal pollution. Even more, the projected evolution of water demand on this mountain range, during the current century, estimates large uncertainties in water supply, which stresses the need for adaptation strategies. In this context, a practical research aim is needed in order to properly manage and conserve these threatened ecosystems.

Considering these needs, this thesis has selected as a case study a Pyrenean headwater stream that presents a hydroelectric dam and that receives the runoff of other tributaries draining from an old antimony mine. In this study case, it was assessed **i)** how seasonality, damming and different precipitation regimes modulate the abiotic conditions and the biofilm and macroinvertebrate communities of these ecosystems, **ii)** how the macroinvertebrate community assemblage can act as a bioindicator for the anthropogenic impacts on the ecosystem structure and function of these ecosystems, **iii)** the use of macroinvertebrates as prey for vertebrate predators of these streams in the current changing context, and **iv)** the impacts of metal pollution and damming on the Pyrenean brook newt (*Calotriton asper*).

In ***Chapter 1***, during a two years span, it was determined, through the analysis of the biofilm and macroinvertebrate communities of a Pyrenean headwater stream, the response of the variables linked to the structure of those communities to seasonality, the presence of a dam, different precipitation regimes, and to metal pollution. The structural metrics analysed followed a strong seasonal pattern that was altered by damming and reduction in the precipitations, due to the water flow reduction and temperature increase that they implied. This promoted an algal and macroinvertebrate biomass increase at expenses of their biodiversity, a situation that is expected to worsen in the current climate change context. The water flow reduction during the dry precipitation regime increased the concentration of metals and metalloids in the stream, but their presence downstream of the dam was lower due to the water diversion that it implied. These results suggest that the maintenance of a sufficient ecological flow in dam-impounded streams is needed in order to overcome the analysed impacts in the current climate change context. Moreover, they highlight the need of more field studies to assess how multiple anthropogenic stressors interact and threaten the ecosystem integrity of headwater streams.

In ***Chapter 2***, the macroinvertebrate community of the stream was used as a bioindicator for the analysis of the alteration of ecosystem. The effects of seasonality, damming and different precipitation regimes were assessed on multiple ecosystemic functions indirectly calculated using taxonomic data of the macroinvertebrate community. The water flow reduction caused by damming and a decrease in precipitations promoted the autotrophy of the stream, altering the normal seasonal pattern that structures the macroinvertebrate assemblage, decreasing its biodiversity and disrupting the proportion of feeding roles. This structural change translated into an impact on the ecosystem functioning of the stream, as it caused an alteration of the direction of the trophic chain. The intensification of bottom-up trophic processes through the autotrophy promotion reduced the strength of top-down processes, decreasing the predator abundance.

In ***Chapter 3***, we analysed the diet overlap between three vertebrate insectivorous species emblematic of Pyrenean headwater streams: *Galemys pyrenaicus*, *Neomys fodiens* and *Cinclus cinclus*. This diet overlap implied a trophic competition situation in which the Pyrenean desman *G. pyrenaicus* specialized on energetically profitable and highly available rheophile prey due to its physiologic and morphologic adaptations that allow it to capture aquatic prey more efficiently, while avoiding terrestrial individuals. This makes this species highly vulnerable to a decrease in food availability and to anthropogenic stressors that reduce the habitat quality. The obtained results show the importance of the habitat, indicating the need to maintain enough riffle sections in the stream to provide of enough quality habitat and prey to the Pyrenean desman that would allow it to effectively use its adaptations. In this regard, it is considered that the flow reduction and connectivity loss that damming causes is a serious threat for the conservation of this species, which needs of a higher awareness. In this study it was also determined that diet analyses are effective in the assessment of the food resource requirements of endangered species such as the Pyrenean desman, but they need to consider the ecology of the prey that it feeds on in order to extract reliable conclusions in prey electivity analyses.

In ***Chapter 4***, a new population of the endangered Pyrenean brook newt, *Calotriton asper*, was described, and the impacts of damming and metal and metalloid pollution on it were assessed. Density of individuals, body sizes and chytridiomycosis prevalence were compared between subpopulations at each side of the dam. It was determined that metal pollution caused a decrease in both the density of individuals and the body size of the upstream subpopulation. No individuals affected by chytridiomycosis were found in this stream despite being only 8 Km apart from an infected population. More research is needed on the impacts of metals and metalloids and damming on *C. asper* in order to manage small populations in headwater streams affected by these anthropogenic threats, improving their conservation.

The results obtained in this thesis provide, in a conservation context, new information about the interactive impacts of climate change, damming and metal pollution on the ecosystem structure and function of the aquatic communities in headwater streams. It was observed that a reduction in precipitations had a synergistic impact with damming through the reduction of the water flow, which promoted the autotrophy of the stream and reduced its biodiversity, altering its ecosystem functioning. These results provide scientific evidence for the proposal of effective management actions for these ecosystems, such as the maintenance of an environmental flow, especially when considering additional local threats to each headwater stream such as metal pollution. The consequences of these interactive threats are especially relevant for key headwater species. On one hand, the availability of basal resources can be impaired, increasing the competition for certain preys on higher trophic levels, displacing species such as the endangered Pyrenean desman (*Galemys pyrenaicus*). On the other hand, the interaction of damming and metal pollution can severely affect the health and distribution of populations of sensitive species such as the Pyrenean brook newt (*Calotriton asper*). This thesis highlights the fact that integrative approaches, including trophic interactions, are useful to understand the effects of anthropogenic impacts on the biodiversity of headwater streams and to propose effective management and conservation actions.

General introduction

1. Ecology of headwater streams

Headwater streams are a unique type of lotic environment, characterized by low water temperatures, distinct seasonal and daily flow variations, oligosalinity and singular hydrological and morphological conditions (Bona et al., 2008). Streams that are considered headwaters are generally of first- and second-order. First-order streams might be intermittent or perennial, but do not have any tributaries, while second-order ones are created by the confluence of two first-order streams (Leopold et al., 1964). Headwater streams are a key component of whole river basins, and they compose over two-thirds of the total length in a river network. Their geomorphology and chemistry play an important role at these larger scales (Figure 1), so their alteration is a matter of concern for the proper management and conservation of whole river basins (Freeman et al., 2007). Consequently, there is an urgent need for a deep understanding of the ecosystem structure and function of headwater streams, and of their vulnerability to anthropogenic stressors.

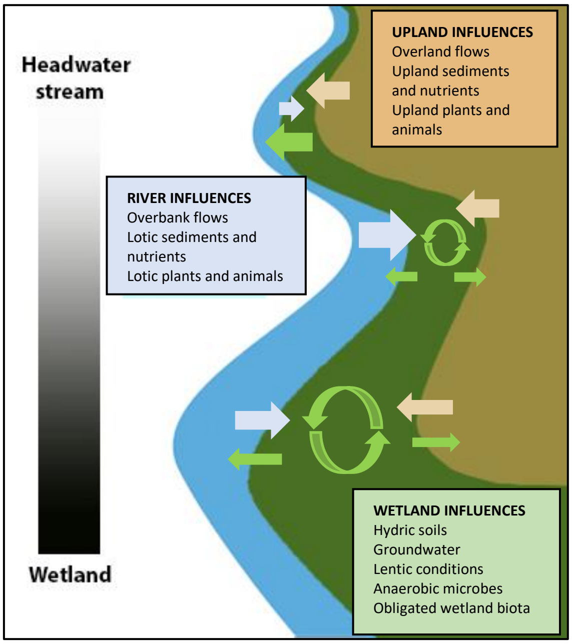

Figure 1. A floodplain continuum diagram that describes the progressive changes in abiotic and biotic conditions along a river floodplain, from headwater streams (top) to mid-river (middle) and to wetlands (bottom). Horizontal straight arrows indicate directional ecotone interactions between adjacent habitats (river-wetland-upland), with wide arrows denoting strong interactions and narrow arrows denoting weak interactions. Circular arrows are internal wetland processes within the floodplain, being its size the reflection of their relative importance. Adapted from Batzer et al. (2018).

A classical theoretical framework used to understand the natural dynamics of streams and rivers was proposed by Ward and Stanford (1989), in which lotic systems are analysed in four different dimensions that interact in a heterogenic way. The first dimension is the longitudinal one, which integrates the upstream-downstream linkages and, thus, allows the energy and matter transfers through the continuum of the stream. Moreover, it is key for animal migration as well as for the drift of plants and immobile organisms. The second one is the lateral dimension, where this energy and matter exchange occurs between the stream channel and the adjacent floodplain system. Alongside the longitudinal one, this dimension is key for animal migrations. The third dimension is the vertical one, which considers the interactions between the channel and the contiguous groundwaters, and has an important role in the stream productivity due to the higher levels of nitrogen and phosphorous present in the groundwater. The last considered dimension is time, superposing a temporal hierarchy over the other dimensions, which scale depends on the phenomenon or organisms investigated. The connection of streams with their adjacent riparian zones generates floodplains, ecosystems with high biodiversity and productivity that have a great economic and cultural importance, but are highly threatened and exploited (Tockner and Stanford, 2002; Argiroff et al., 2017; Batzer et al., 2018).

Floodplains from headwater streams are narrow, and their major contribution to the ecosystem structure and function of headwaters comes from the trees adjacent to the stream, which provide shading and an important organic matter input through the leaves that fall on the water. On the other direction, the influence of the channel on the floodplain is weak due to overbank flooding being rare and unpredictable (Figure 1). Nonetheless, the connectivity that this open system generates has an important influence over the assemblages of fish, amphibian, macroinvertebrate and macrophyte communities (Argiroff et al., 2017; Batzer et al., 2018). If we aim to comprehend the dynamics of headwater streams and the consequences of anthropogenic disruption on their interactive pathways, as well as to effectively manage the biota in these systems, it is mandatory to apply a broad spatio-temporal perspective in the research of these open ecosystems and to consider their unique and changing characteristics (Freeman et al., 2007; Batzer et al., 2018).

A frequent method used to understand the characteristics and dynamics of streams is the study of their biotic and abiotic structural components, which also allows for the understanding of their ecological condition and water quality (Hopkins et al., 2010; Hornbach et al., 2016). This structural approach consists on decomposing and describing the environmental heterogeneity and its associated selective forces into different natural scales as a hierarchical description. This is widely used to understand the characteristics of ecosystems at particular temporal or spatial scales by isolating processes at a single level (Biggs et al., 2005). The functions of an ecosystem are derived from its structure as processes of exchange of matter and energy between its biotic and abiotic components (Freeman et al., 2007). The most relevant of these processes in streams are primary and secondary production, respiration, nutrient turnover, decomposition, processing of decaying organic matter, direction of the trophic chain, and resistance and resilience of the ecosystem in front of disturbances. Because these metrics are strictly linked to the structural components and integrate measures of physical, chemical and biological activity, they provide additional information to structural measures about the ecosystem condition. Therefore, ecosystem structure and function measures are considered complementary assessments of biotic responses to environmental conditions, not redundant, since the utility of each individual metric may vary depending on the stressor. Consequently, stream function measures do not always show unidirectional responses to stress, hindering the prediction of changes (Sabater and Tockner, 2009). In order to understand the ecological dynamics of headwater streams, their biotic and abiotic structural components will be next considered and defined.

1.1. Biotic components of headwater streams – Biodiversity and bioindicators

Biodiversity is a concept that refers to the variety within and among living organisms and their communities and processes. It can be measured with many approaches and scales, such as the number and identity of species, their assemblage and interactions, and the processes derived from them (Swingland, 2001). This integrative approach focuses on biological communities, which is the assemblage of interacting species in a determined habitat and the interrelationships that bind them (Putman, 1993). The study of biological communities is not only important for the structural and functional characterization of ecosystems, but also for the assessment of their environmental health due to their close relationship with the habitat conditions. Biodiversity loss compromises the components of these communities, negatively affecting the correct functioning of the ecosystem. Not all species contribute equally to the healthy functioning of ecosystems, which pushes arguments in favour of biodiversity conservation (Dudgeon, 2010; Catford et al., 2013). The species that have a disproportionately greater effect on the ecological processes, and whose loss would result in significantly greater consequences for other species and their interactions, are considered key species.

Headwater streams and their biodiversity depend on the primary and secondary production that the biofilm and macroinvertebrate communities generate, which can be modulated by multiple key species at higher trophic levels through top-down processes. An example of this assemblage can be seen in the headwater streams of the Pyrenees mountain range, which is the ecological context that this thesis is focused on. In these ecosystems, vertebrate key species such as the Pyrenean brook newt (*Calotriton asper*), the Pyrenean desman (*Galemys pyrenaicus*) and the river trout (*Salmo trutta fario*) regulate the macroinvertebrate community that feeds on the periphyton, having a great effect on the matter and energy transfer of the ecosystem (Figure 2). The loss of key biotic components in headwater stream communities can severely compromise the functioning of these ecosystems and, thus, the assessment of anthropogenic impacts on the structural components of headwater streams should be a priority in the conservation of these environments.

1.1.1. Bioindicators as a tool for the conservation of headwater streams

Biodiversity in headwater streams is highly vulnerable to many human-mediated processes and stressors due to the singular and fragile conditions of these ecosystems. The main threats to these ecosystems are pollution, habitat destruction, flow modification, over-extraction of water and alien species introductions (Revenga et al., 2005; Dudgeon et al., 2006; Dudgeon, 2010; Ormerod et al., 2010). As mentioned previously, biodiversity is crucial for the correct functioning of ecosystems: it allows communities to efficiently capture resources, produce biomass and recycle essential nutrients (Cardinale et al., 2012), increases the stability of ecosystem processes through time (Hector et al., 2010; Campbell et al., 2011) and buffers ecosystems against environmental variability. The species and communities that conform the biodiversity of a habitat, as well as the biological processes that are derived from it, can be used to assess the quality of the environment and how it changes over time. This approach defines each biotic component of the structure of an ecosystem as a bioindicator (Holt and Miller, 2010). Most aquatic organisms respond differently to a certain environmental condition or stressor (Unrine et al., 2007; Wassens and Maher, 2011; Maes et al., 2018), and no group of organisms or biological metric is always the best suited for the assessment of a specific disturbance. In order to obtain a comprehensive signal of ecosystem change, the use of several groups as bioindicators should be promoted, as they provide complementary perspectives of ecological integrity (Wan Omar, 2010; Hopkins et al., 2011). In this regard, considering the biodiversity of headwater streams and its conservation, the characterization of their main structural components, as well as their potential use as bioindicators, must be defined.

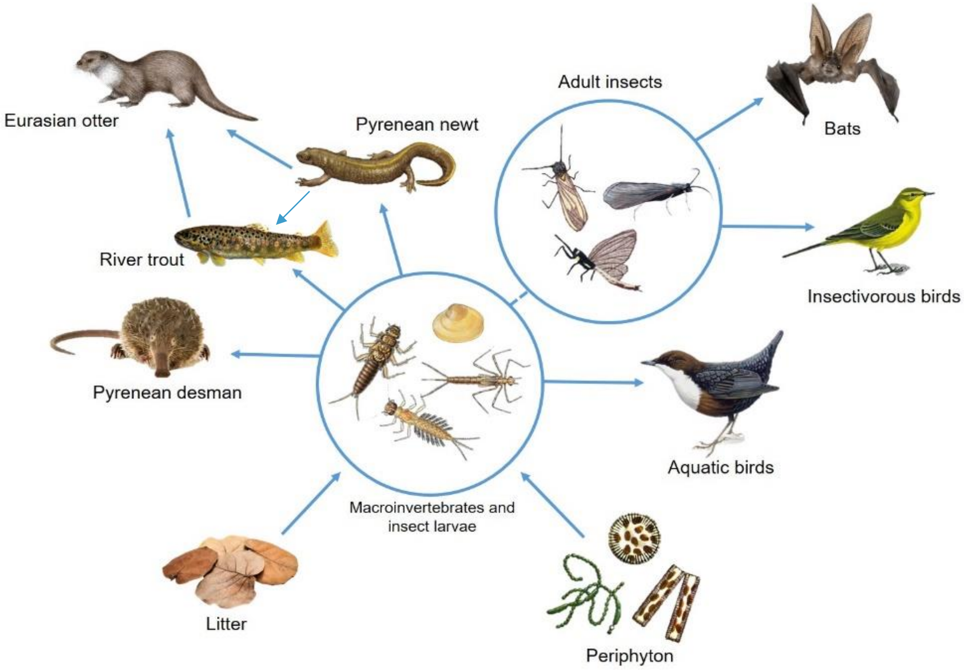

Figure 2. Diagram of biotic interactions in a headwater stream from the Pyrenees. Adapted from Limnopirineus (2019). Arrow direction indicates that one group is consumed as a resource by another group.

1.1.1.1. Fluvial biofilms

Fluvial biofilms (or periphyton) are complex biological structures composed by an autotrophic component, containing mainly green algae, diatoms and cyanobacteria, and a heterotrophic component, containing mainly bacteria, archaea, viruses, fungi and protozoans. Both components are located in close physical contact and embedded in a mucopolysaccharide matrix conformed of extracellular polymeric substances (EPS matrix) (Lock et al., 1984; Romaní, 2010; Mora-Gómez et al., 2016) (Figure 3). These biological systems play a crucial role in the energy flow and nutrient cycling of headwater streams (Bona et al., 2008; Wan Omar, 2010). They integrate the information of environmental conditions due to the stickiness of the EPS matrix, which traps and accumulates particles and nanoparticles from the water, and due to physicochemical mechanisms that adsorb cations, anions and apolar compounds (Flemming and Wingender, 2010; Huang, 2014). Biofilms play a key role in the primary production of the ecosystem (Guasch and Sabater, 1998) and in its organic matter processing (Romaní and Sabater, 2001). The sensitivity of fluvial biofilms to environmental conditions allows the use of many characteristics of the biofilm community structure as bioindicators, such as the species composition, cell density, cell biovolume, and ash free dry mass and chlorophyll-a contents (Hillebrand et al., 1999; Berthon et al., 2011; Corcoll et al., 2012). Biofilm communities are widely used as bioindicators due to the fact that they are fixed (Li et al., 2010), have a relatively quick recolonization time after perturbations in water, are easy to sample, are widespread and common (Biggs, 1989), and changes in water quality and in the physical structure of the stream affect their species composition (Bona et al., 2008).

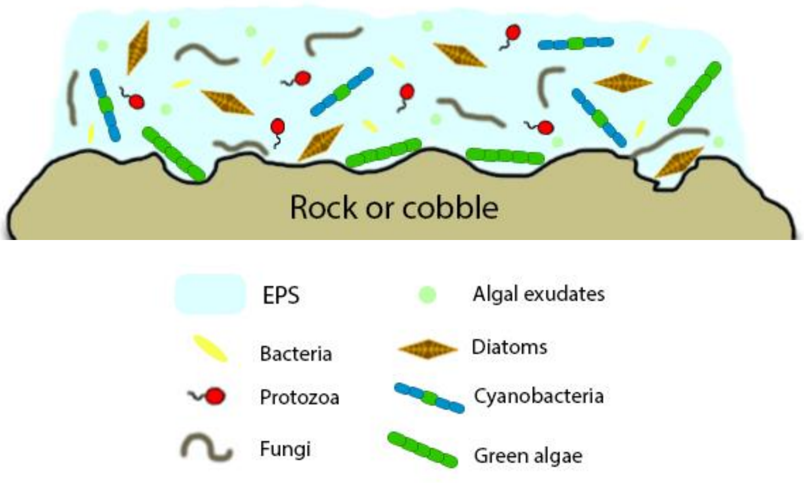

Figure 3. Structure of a freshwater biofilm growing over a rock or cobble. Adapted from Mora-Gómez et al. (2016).

1.1.1.2. Benthic macroinvertebrates

Benthic macroinvertebrates are responsible for the primary processing of organic matter in headwater streams, generating its secondary production. This organic matter can be autochthonous if it comes from algae, plants and biofilms of the benthos, or allochthonous if it comes from the surrounding riparian and upland vegetation. Macroinvertebrates can even process the decomposing organic matter of the stream, acting as detritivores (Cummins, 2016). The secondary production that this community generates constitutes a key link between the energy sources of the stream and its top predators by acting as prey (Allan, 1995; Li et al., 2010). Because of this intermediate position in the trophic chain and their sensitivity to environmental change, benthic macroinvertebrates are the most cited group for bioassessment of aquatic ecosystem health (Rosenberg and Resh, 1993; Barbour et al., 1999; Resh, 2008). Macroinvertebrate community assemblages are constituted of many species with a wide range of trophic levels, pollution tolerance and lifespans, integrating the impacts of both short-term and long-term environmental stress and their cumulative effects (Li et al., 2010; Clarke et al., 2003). Despite this, it is known that in systems affected by multiple stressors, macroinvertebrate community measures alone may not completely explain the patterns of water quality (Hopkins et al., 2011), needing complementary assessments with other bioindicator groups. For example, biofilms are more affected by the local habitat than macroinvertebrates, which depend mainly on the general condition of the stream (Bona et al., 2008). Macroinvertebrates have several advantages as bioindicators for research and environmental management objectives as they present a wide distribution, a positive cost-efficiency relation (Bonada et al., 2006), and a large-scale applicability, but they have a major drawback: the structure and function of their communities change seasonally and with the location within the basin (Álvarez-Cabria et al., 2010).

1.1.1.3. High trophic levels as key species: vertebrates

The structure and function of aquatic food webs, and their energy flow, can be mediated in two control directions. *Top-down* control takes place when consumers and predators regulate the abundance of their prey at lower trophic levels, while in *bottom-up* control the abundance of consumers and predators is limited by the availability of basal resources. *Top-down* control can be exerted by vertebrates in headwater streams, as they usually stand at the top of the food chain, but it can also be exerted by macroinvertebrates that regulate the algal and biofilm communities, which are the main groups that drive the *bottom-up* control. In any of these processes, certain groups and species are considered key as they are one of the main components that allow the correct structuring and functioning of the ecosystem (Nery and Schmera, 2016). Key species, which have a great effect on ecological processes, can have an important influence on the direction of the energy flow of an ecosystem. Consequently, assessing the interactions between key species and their environment is mandatory for a correct understanding of the structure and function of ecosystems.

The coexistence of multiple species in the same ecosystem implies a niche differentiation in their habitat or prey use, which, under limiting conditions, may lead to the competitive exclusion of the weaker competitor. In threatened ecosystems, many disturbances can cause a shift in resource availability, exacerbating the interactions between those competitors. Because of this, understanding the resource use and niche overlap between key species with conservation concerns is crucial for the determination of their vulnerability to stressors (Wisz et al., 2013; Biffi et al., 2017). Under this context, the assessment of the state of populations of key species from the high trophic levels of Pyrenean headwater streams can be a useful tool to assess the anthropogenic impacts and conservation needs of these ecosystems. In this regard, species that are known to have a key role in the energy and matter transfer processes of Pyrenean headwater streams have been selected to assess the previously mentioned needs of these ecosystems.

1.1.1.3.1. Newts and salamanders – Calotriton asper

Newts and salamanders are key structural components of the matter and energy flow in aquatic food webs because of their intermediate position, acting as prey, predators and competitors at the same time. They drive a *top-down* control through the predation on the macroinvertebrate community, but they can also be a food resource for higher trophic levels (*bottom-up* control) such as fish, snakes and mammals. Due to their amphibian life cycle, they also suppose a link between terrestrial and aquatic ecosystems by three different levels of lateral connectivity: (1) the consumption of allochthonous food resources in aquatic systems, (2) the consumption of newts and salamanders by terrestrial predators and (3) direct movements of newts and salamanders between habitats (i.e., seasonal migrations with breeding purposes and emergences of metamorphosed larvae) (Sánchez-Hernández, 2020). Because of this dependence of both terrestrial and aquatic habitats, plus the fact that their highly permeable skin makes them sensitive to water pollutants (Zhou et al., 2008), amphibians are an ideal group to assess anthropogenic impacts on headwater streams. Characterization of the amphibian populations are especially important when managing these environments, and specific plans for their monitoring and management should be prioritized when endangered species or important populations are present (Montori et al., 2007). In this regard, the emblematic and endangered Pyrenean newt *Calotriton asper* (Dugès, 1852) should be considered when assessing the ecological integrity of Pyrenean headwater streams (Montori, 1988; Sánchez-Hernández, 2020). The larval and adult life stages of this species are totally aquatic, but the subadult is terrestrial (Montori, 1988), which also contributes to the lateral connectivity of headwater streams with the adjacent land.

1.1.1.3.2. Fish community – *Salmo trutta fario*

Another key component of the ecosystem structure of headwater streams that can also be used as bioindicator is the fish community, which has been used for river health monitoring for many years (Li et al., 2010). They are good indicators of water quality due to their sensitivity to pollution, long-life cycle, high mobility, and trophic position as top predators (Barbour et al., 1999; Naigaga et al., 2011; Li et al., 2010). Their sensitivity to the environmental health makes the fish community structure to respond significantly to anthropogenic disturbances (Li et al., 2010). Focusing on Pyrenean headwater streams, the river trout *Salmo trutta fario* (Linnaeus, 1758) is a key species that competes with other vertebrate species for the macroinvertebrate community as a food resource and can also prey on other key organisms such as the Pyrenean brook newt (Montori, 1988; Muñoz-Mas, 2018). This species is a well-known bioindicator in headwater streams due to its cold water needs and its sensitivity to rising temperatures, which makes it vulnerable to the current global temperature increase caused by climate change, among many other threats such as damming or pollution (Muñoz-Mas, 2018).

1.1.1.3.3. Aquatic mammals – *Galemys pyrenaicus*

Galemys pyrenaicus (E. Geoffroy Saint Hilaire, 1811, Talpidae) is another key species of the Pyrenean headwater streams that has an important role in the matter and energy flow of these ecosystems through the consumption of macroinvertebrates. Because of this, it competes with other high-trophic level predators like the brown trout, the Pyrenean brook newt, the white-throatted dipper (*Cinclus cinclus*), and the Eurasian water shrew (*Neomys fodiens*). *Galemys pyrenaicus* is usually found coexisting with *N. fodiens* and *Cinclus cinclus* in Pyrenean headwater streams in a competitive environment, as these three species use ephemeropterans, trichopterans and plecopterans as staples in their diets, but each predator has a preference for certain groups due to their own adaptations to capture prey (Bertrand, 1994; Castién and Gosálbez, 1999; Biffi et al., 2017a).

In this regard, *Galemys pyrenaicus* can easily predate on highly digestible macroinvertebrates because of its diving adaptations (Biffi et al. 2017a), thus feeding on energetically profitable prey. Nonetheless, the influence of prey, competitors, and predators on the survival of this species with conservation concerns, and how anthropogenic stressors modulate them, still lacks knowledge (Aymerich and Gosàlbez, 2015; Biffi et al., 2017a). This mammal is an endemism of the Pyrenean headwaters and the northern Iberian Peninsula, and its populations suffer alarming declines (Charbonnel et al., 2016). Because of this, it is used as a flagship species by encouraging local, national and European conservation initiatives (Aymerich and Gosàlbez, 2015), meaning that it is used as the focus of a broader conservation campaign (Veríssimo et al., 2011). Due to the vulnerability of this species, the state of the populations of *Galemys pyrenaicus* and their response to the availability of food resources could act as bioindicators of the ecological integrity of Pyrenean headwater streams.

1.2. Abiotic components of headwater streams – Seasonality of the water flow

The water availability and quality of headwater streams is strongly influenced by seasonality. The Pyrenees mountain range, in the Mediterranean basin, is a great example of this situation because they present a marked spatial and temporal variability in the precipitation regime, and are influenced by contrasted humid and dry seasons (Beguería et al., 2003; Serrano-Mueia et al., 2008; López-Moreno and Beniston, 2009a; Sanmiguel-Vallelado et al., 2017; OPCC-CTP, 2018). Seasonality on Pyrenean headwater streams is driven by snow dynamics that regulate the water flow with the timing of the snow accumulation and the snowmelt. Snow serves as a water reservoir during winter, accumulated as solid precipitation, and generates high water flows during the snowmelt on spring and early summer, a contribution larger than the one that rainfall provides in most Pyrenean basins. This seasonal pattern causes low and uniform flows during winter and greater and fluctuant flows during spring, with high interannual variabilities (Sanmiguel-Vallelado et al., 2017). The ecosystem structure and function of headwater streams are strongly influenced by these seasonal and interannual variations in water flow (Biggs et al., 2005).

2. Anthropogenic stressors and threats to headwater streams

Streams and rivers are among the most vulnerable, yet exploited and threatened, ecosystems of the planet (Loh et al., 2005). They are critical for water supply, sanitation, and for the support of livelihoods of between 1.5 and 3 billion people (MEA, 2005). Their integrity is being threatened by the combined effects of pollution, channelization, damming, water abstraction, species introductions and riparian simplification, among others (Bona et al., 2008; Sabater and Tockner, 2009). These alterations cause changes in the composition of the biotic community of the stream, usually with a simplification and impoverishment of its biodiversity (Maddock, 1999), reducing its capacity to recover from disturbances and affecting the ecosystem functioning (Bona et al., 2008; Sabater and Tockner, 2009). These impacts have been historically concentrated in areas with major anthropic presence, but in recent times, headwater streams have started to become seriously threatened by the influence of human activities (Bona et al., 2008). Being impacted by multiple stressors at the same time, it is not always possible to discern which human alteration is the most detrimental for a stream ecosystem (UNEP, 2007). Stressors may act at different biological levels (Figure 4), and can interact exacerbating or mitigating the effects of some of them, resulting in unexpected effects (Gessner and Tlili, 2016).

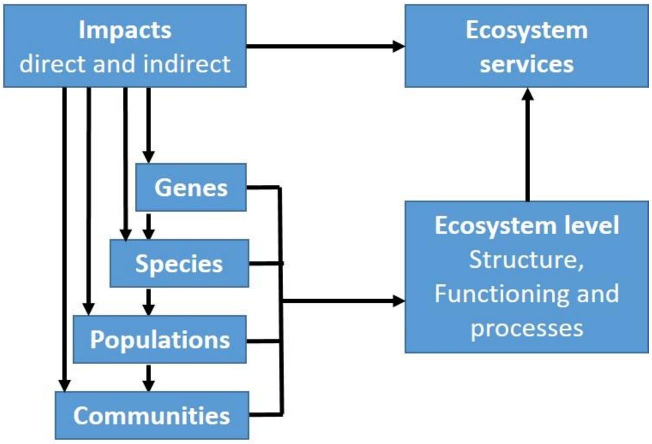

Figure 4. Flow diagram showing how ecosystem components are linked under direct and indirect impacts from stressors. Adapted from UNEP (2007).

2.1. Stress ecology

Stress is defined, in an ecological context, as the internal state of an organism resulting from placing it outside of its ecological niche. This phenomenon goes through different phases during which, if the initial exposure to a stressor is not strong or fast enough to kill the organism, it will activate defence mechanisms in a resistance phase until the exhaustion causes a collapse of vital cellular functions (Steinberg, 2012). In this context, a stressor is often associated with pollution or other detrimental pressures, but adverse situations for a certain organism can be favourable for another (Piggot et al., 2015). Within an ecological perspective, multiple natural and anthropogenic stressors can act at the same time on biotic communities, which can cause different types of responses: additive, if the combined effects of the analysed stressors equal the sum of their individual effects, or multiplicative, if the resultant effect is greater (synergism) or lesser (antagonism) than the sum of their individual effects (Piggot et al., 2015; Jackson et al., 2016). The biological response of organisms to stressors are indicators of stress, which can be expressed as growth changes, modifications in the food-web structure, an increase in disease susceptibility, morphological abnormalities, a reduction in the fecundity and even as mass mortality events, among others (Steinberg et al., 2012). Consequently, understanding and monitoring the responses of biological communities to stressors is key in order to properly manage and conserve ecosystems affected by natural and anthropogenic threats.

2.2. Impacts of concern on the conservation of headwater streams

2.2.1. Damming

The United Nations are pushing measures to provide affordable and reliable access to electricity for more than 940 million people that do not have access to it, especially in low-income regions (The World Bank, 2018a; The World Bank, 2018b). One of the main strategies that are being promoted is the construction of dams in order to generate renewable electricity (Zarfl et al., 2019). 22% of the world's electricity currently comes from renewable sources, 73% of which is hydropower (The World Bank, 2018a). After the 21st Conference of the Parties (COP21), the decision organ of the United Nations Climate Change Conference, in Paris 2015, many governments decided to promote the construction of hydropower structures to address climate change and its consequences on terrestrial and freshwater. This derived in the current planning or construction of more than 3.700 medium and large hydropower dams around the world, doubling the current installed capacity (Zarfl, 2015).

Hydropower reservoirs do not only increase the security of energy supply, they also serve as recreational areas, provide flood protection and drinking water, and can be used for aquaculture and irrigation purposes (Berga, 2016), making the expansion of hydropower a potential solution to multiple social and economic challenges at the same time (Zarfl et al., 2019). A total of 52% of the surface area connected by worldwide large river systems (considered with a discharge higher than $350m^3/s$) is heavily modified. It has been estimated that about 15% of world's total runoff is retained by around 45.000 dams higher than 15m, which makes a total of $40.000km^3/year$. From this volume, a 10% is abstracted and up to 6% is evaporated. Europe contains the highest fraction of altered river segments, and its water withdrawal is projected to rise from $415km^3$ to $660km^3$ by 2070, which will cause the European area affected by severe water stress to increase from 19% to 36%. This water scarcity stress is already a characteristic phenomenon in Mediterranean areas (Sabater and Tockner, 2009).

Despite all their social and economic benefits, dams have many environmental downsides, including serious and long-term effects: habitat degradation and fragmentation, biodiversity loss, erosion of ecosystem services, changes in hydrology and sediment transport, flow reduction, water temperature increase, deterioration in water quality and the spread of water-associated diseases and invasive species (Bednarek, 2001; Lessard and Hayes, 2003; Zarfl, 2019). If we aim to conserve, manage and, if needed, restore streams and rivers affected by hydropower impoundments, while at the same time assuring goods, services, and energy to human populations, we need to assess how these impoundments can compromise the structure and function of the lotic bodies where they are placed.

2.2.1.1. Water flow reduction

The water flow of a stream is an interactive consequence of precipitation, climate, land use and geomorphology (Ceola et al., 2013), and it is considered a master variable that structures the habitat, drives the quality of the water, and controls population and community dynamics on stream ecosystems. Water flow regimes control the channel morphology and its size, habitat diversity and substrate stability, and drive processes that influence the abundance, distribution and diversity of organisms. Each stream can vary widely in the magnitude and regularity of its water flow, which determines the characteristics of its physical environment and biotic community. Since stream communities are adapted to natural variability of the water flow, its anthropogenic alteration supposes a threat to the stability and correct functioning of these ecosystems (Bednarek, 2001; Mor et al., 2018). Physical obstacles like dams and weirs alter the hydrological patterns and natural flow regimes in different ways, depending on the hydrology of the stream and on the purpose of the reservoir and its size (Bednarek, 2001; Nilsson et al., 2005). The structure and function of lotic ecosystems can be directly impacted by a water flow reduction at both sides of the reservoir as well as indirectly impacted through to changes on the physical and chemical parameters of the water (Sabater and Tockner, 2009) (Table 1).

2.2.1.2. Connectivity loss

Connectivity is a term used by freshwater ecologists to describe spatial linkages within rivers and streams (Freeman et al., 2007). A classical approach to connectivity in this context is defined by Ward (1997), who defines riverine connectivity as the energy transfer across the riverine landscape. This connectivity definition can be encompassed in a broader approach together with the hydrologic cycle, defining the hydrologic connectivity, which refers to the water-mediated transport of matter, energy and organisms within or between elements of the hydrologic cycle, considering a broader scale beyond the watershed (Pringle, 2001; Tockner and Stanford, 2002). Hydrologic connectivity is crucial for the maintenance of the quality, temperature and sediment transport of the water flow, as well as for the migration of organisms throughout the riverine system (Westra et al., 2000; Larsen et al., 2019). Obstacles in the river corridor, like dams, create physical and thermal obstructions for migrating and drifting stream organisms, isolate populations and habitats, and disrupt interactions between freshwater, terrestrial and coastal systems (Bednarek, 2001). In headwater streams, impoundments unavoidably affect the ecosystem structure and function locally, but also at larger scales through the loss of unique functions to downstream and upland systems (Freeman et al., 2007).

2.2.1.3. Impacts on the ecosystem structure and function

Damming impacts stream systems at different scales by altering runoff patterns, fluxes to downstream reaches and eliminating distinctive habitats, which has several effects on the ecosystem structure and function at local and regional scales (Freeman et al., 2007). The decrease of water flow caused by damming leads to an increased exposure to UV radiation, higher water temperature variability and higher concentrations of nutrients and pollutants, which can lead to cascading effects on the biodiversity of the ecosystem and its functioning. This may decrease the efficiency of many ecological processes and enhance the Gross Primary Production (GPP) of the stream, favouring a shift from natural heterotrophy towards an altered autotrophic state, even in relatively pristine rivers (Sabater and Tockner, 2009).

2.2.2. Climate change

The Mediterranean Basin is one of the most vulnerable regions to climate change. Most models indicate that stream ecosystems in Mediterranean regions will be affected by altered flow regimes, increased frequency and magnitude of floods, unexpected droughts (Daufrense and Boët, 2007; EEA, 2008; Sabater and Tockner, 2009), and increased water temperature (Caissie, 2006; Hester and Doyle, 2011; Moss, 2012; van Vliet et al., 2013). Understanding the effects of climate change on wildlife populations, their community assemblage, and on the functioning of their ecosystems is one of the main challenges of modern ecology (Daufresne and Boët, 2007).

Table 1. Indirect effects of multiple physical and chemical parameters, caused by a low water flow, on the structure and function of stream ecosystems. Adapted from Sabater and Tockner (2009).

Parameter	Effects on low flow	Affected biotic group	Affected ecosystem parameter
Temperature	*· Lower oxygen content* *· Higher metabolic rate* *· Combined effects with toxicants and nutrients*	*· All groups*	*· Decrease in oxygen availability* *· Higher primary production* *· Higher respiratory rates* *· General effects on structure and metabolism*
Conductivity	*· Enhanced water salinity*	*· All groups*	*· Physiological regulation* *· Changes in community composition*
Organic matter	*· Accumulation of organic matter*	*· Primary producers and bacteria*	*· Slower decomposition* *· High oxygen consumption* *· High mineralization*
Sediments	*· Siltation*	*· Primary producers, invertebrates and fish*	*· Reduced production* *· Changes in community composition* *· Difficulties in gas diffusion*
Nutrients	*· Higher concentration; eutrophication*	*· Primary producers, with potential bottom-up effects*	*·Higher gross primary production* *· Lower efficiency on materials processing*
Pollutants	*· Increase of pollutant concentration*	*· All groups, with complex food web effects*	*· Biodiversity decrease* *· Effects on metabolism* *· Effects on material processing*

An appropriate region to study how climate change affects the ecosystem structure and function of headwater streams are the Pyrenees. Climate on this region is highly complex and depends on the spatial and seasonal variability related to the Atlantic-Mediterranean gradient of its range (López-Moreno and Beniston, 2009a). Currently, average temperatures are increasing between 0.25 and 0.36°C per decade in this mountain range, and its water availability is decreasing due to a reduction in the annual pluviometry of around 2.5% per decade, especially in the southern slope (López-Moreno et al., 2008a, 2008b, 2009b; Batalla et al., 2018; OPCC-CTP, 2018). Moreover, the Pyrenees present a high interannual variability in the precipitation levels. This can be observed in Figure 5, which indicates the data of the average water flow in the Ter River at the Pyrenean location of Sant Joan de les Abadesses. An increase on the frequency of droughts (particularly relevant on spring and summer) and high precipitation events, as well as a reduction in the snowyield and an earlier snowmelt, are also being reported (López-Moreno and

Beniston, 2009a; Sanmiguel-Vallelado et al., 2017), which will have overwhelming effects on the biodiversity of this region (García de Jalón et al., 1986; Carvalho et al., 2010; De Pous et al., 2016; Biffi et al., 2017).

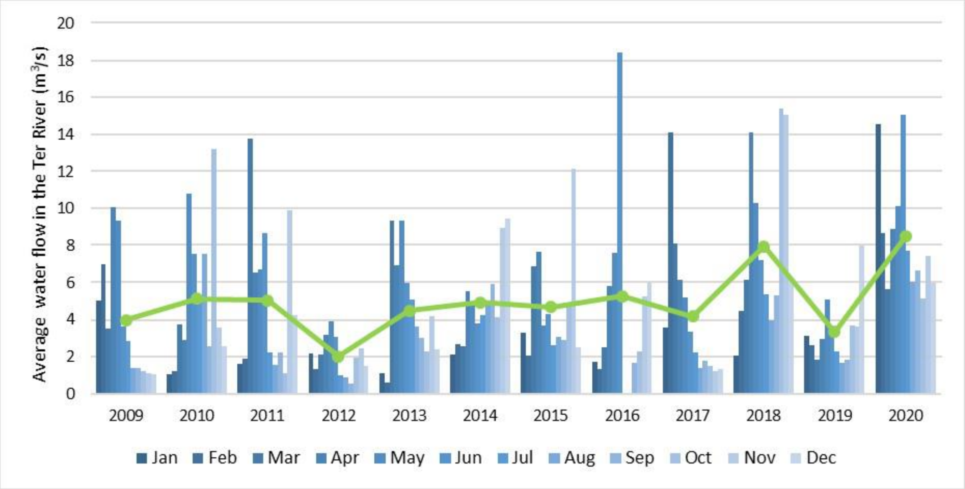

Figure 5. Average values of water flow of the Ter River at Sant Joan de les Abadesses during the period 2009-2021 for each month (blue columns) and each year (green line).

2.2.3. *Metal and metalloid pollution*

Multiple metals and metalloids (from now on both type of chemicals will be referred only as *metals*), essential and not-essential, are potentially harmful for most organisms since they can accumulate until they reach a toxic concentration, which can translate into a considerable ecological damage (Gümgüm et al., 1994). Assessing the environmental impacts of metals is the domain of ecotoxicology (Gessner and Tlili, 2016), which was defined originally by Truhaut (1977) as the branch of toxicology concerned by the effects of pollutants on the constituents of an ecosystem in an integrated context. It has been recognised that the traditional focus of ecotoxicology on individuals is insufficient to assess environmental impacts. Consequently, integrating ecological principles into the design and implementation of ecotoxicological research is essential for the assessment and prediction of pollutant impacts on biological communities and ecosystems (ECHA, 2016). This is especially relevant because chemical pollutants are key drivers of community structure and ecosystem processes (Gessner and Tlili, 2016) through direct impacts on key species as well as through indirect effects emerging when species interactions are disrupted, which can weaken or exacerbate individual pollutant effects (Relyea and Hoverman, 2006; Halstead et al., 2014).

Metals can be naturally found in low concentrations in aquatic systems due to the slow leaching from soil or rocks into water, but anthropogenic activities such as mining, industry development, and agriculture promote the rapid increase of metal concentrations in aquatic systems (Zhou et al., 2008; Guasch et al., 2009; Corcoll et al., 2011). Metals may transform into persistent metallic compounds with high toxicity and bioaccumulate in the organisms, being magnified in the food chain and, thus, threatening the health of ecosystems (Jin, 1992). Multiple bioindicators can be used for the assessment of metal pollution in aquatic ecosystems. Suitable bioindicators for this purpose are expected to have some of the following characteristics: (1) can accumulate high levels of pollutants without dying; (2) live in a sessile style and represent the local pollution; (3) are abundant and widely distributed; (4) live enough to compare between various ages; (5) can be analysed microscopically at tissue or cellular level; (6) are easily sampled and/or raised in the lab; (7) are kept alive in water; (8) occupy an important position in the food chain, and (9) a dose-effect relationship can be easily observed in them (Zhou et al., 2008). Considering this, biofilms, macroinvertebrates, fish, and amphibians are good bioindicators for the evaluation of metal pollution, each by multiple of the described conditions (De Jonge et al., 2008; Zhou et al., 2008). The assessment of the impacts of metal pollution on stream ecosystems is complex, and requires approaches that consider the biological and chemical diversity alongside the natural variability of environmental factors at multiple temporal and spatial scales (Gessner and Tlili, 2016) (Figure 6). Population-level (density, size distribution), community-level (species richness metrics, multivariate analysis of community composition) and bioaccumulation responses of aquatic organisms to metal pollution are strong measure tools for this kind of evaluations (Zhou et al., 2008).

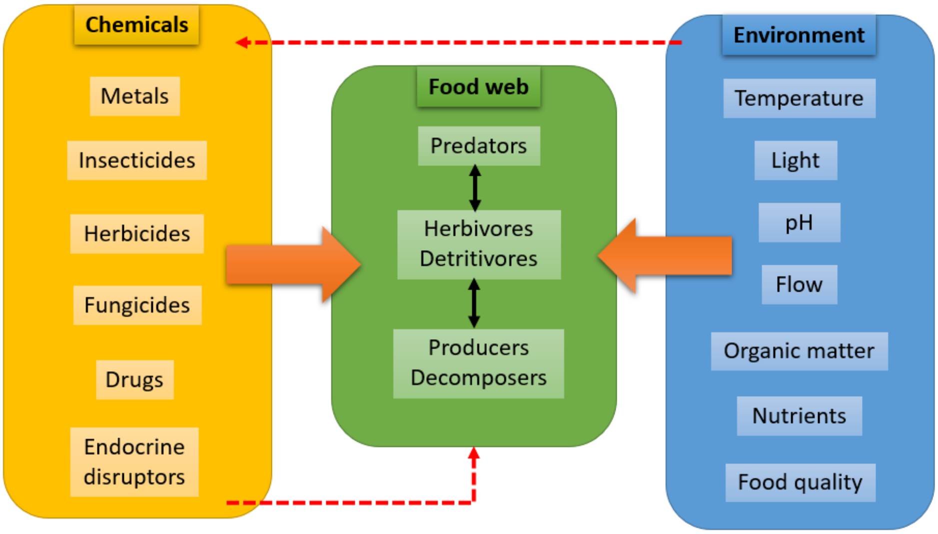

Figure 6. Diagram showing how chemicals and environmental factors act on different levels of food webs. Red arrows indicate the indirect influence of the environment on food webs through its influence on many different chemicals, as it can modify their bioavailability and toxicity on biological communities and food webs. Orange arrows show the direct impacts of chemicals and environmental factors on the food webs. Adapted from Gessner and Tlili (2016).

2.3. Conservation and restoration of streams

Streams that could be considered natural are a rare phenomenon. The recognition of human impacts on streams and the current rise in environmental awareness have driven initiatives of restoration in order to improve their water quality and ecological integrity (Maddock, 1999). For this purpose, it is necessary to define which channel forms and features are pertinent for the maintenance of a natural biodiversity and key ecosystem functions, and at which spatial scales they are relevant. Stream ecosystems can rarely be reverted to a natural state because human societies depend on the natural resources that they provide, like drinking water or space use. However, we should aim to use those resources in a sustainable way while maintaining a stable ecosystem functioning. When considering which stream channel features are needed to maintain in a stream conservation project, there is not a simple answer, which is often the case in nature conservation. Each spatial configuration will benefit some species at the expense of others, increasing some functions at the detriment of others. Therefore, clear goals must be set for the maintenance and restoration of the ecosystem structure and function, which must be translated into specific management goals

(Elosegui et al., 2010). Conservation and restoration plans should include the creation or expansion of freshwater protected areas, development of integrated catchment management plans, dam modification, and environmental flow management (Abell et al., 2007, Arthington et al., 2010, Linke et al., 2011; Liermann et al., 2012).

3. Research needs

Conservation ecology is a discipline which research aims to benefit the ecological integrity of an ecosystem by identifying patterns and mechanisms, quantifying changes, recognising problematics and testing possible solutions. A current problematic in this branch of knowledge is the existing mismatch between the priorities of academic researchers and the needs of conservation practitioners. Because of this, research with a practical aim should be promoted in order to effectively manage threatened ecosystems (Stinchcombe et al., 2002; Linklater, 2003; Knight et al., 2008). Sutherland et al. (2010) identified key questions that, if answered, would have the greatest impact on the practise of conserving biodiversity worldwide. This doctoral thesis will be based under the context of two main aspects of those research needs. On one hand, there is a global need to understand which biotic elements, and in which locations, are the most vulnerable to anthropogenic stressors and how its management should be implemented. To understand the properties of future ecosystems derived from the impacts of climate change and anthropogenic disturbances, the key factors that shape the characteristics of current ecosystems must be known (Catford et al., 2013). On the other hand, focusing on stream ecosystems, it is mandatory to know which freshwater species and communities are most vulnerable to human impacts in a climate change context. As described previously in this introduction, the Pyrenees are a vulnerable habitat affected by many anthropogenic threats and by climate change, and there is a critical need to assess which species of its headwater streams are more vulnerable to those individual stressors and their combination (Dudgeon, 2010).

In this regard, understanding the impacts of damming, pollution and a precipitation decrease on the ecosystem structure and function of headwater streams in the Pyrenees could provide valuable information on the assessment of the previously mentioned practical research needs for an effective conservation of freshwater ecosystems. Current research efforts on the impact of multiple human stressors on headwater streams are increasing, but more field studies are required to understand the effects of each of those stressors and their interaction. Furthermore, the study of these impacts on diverse biotic components is needed, since field studies that integrate multiple groups are still very limited (Kalogianni et al., 2017).

New research is also needed in the field of ecotoxicology. Pollutants interact with numerous environmental factors, and freshwater ecosystems are usually affected by multiple stressors, limiting the power of field studies to attribute ecological effects to specific causes. A key challenge for ecotoxicology is to predict the combined effects of chemicals with environmental stressors on the structural parameters and processes of an ecosystem. Currently, the assessment of ecotoxicological impacts rely in great measure on controlled laboratory assays with one or just a few species as models, which limits the extrapolation of results to complex natural systems. Framing ecotoxicological assessments in an ecological context can provide new knowledge about direct and indirect effects of pollutants on populations, communities and ecosystems, as well as providing new insights into the environmental relevance of the extrapolation of laboratory test results (Clements et al., 2015; Gessner and Tlili, 2016).

General objectives and hypotheses

Considering the current and future threats on the ecosystem structure and function of headwater streams, and the need for practical research that can provide effective management solutions to these threatened habitats and its biodiversity, this thesis aims to use a Pyrenean headwater stream affected by the presence of a hydroelectric dam and metal pollution as a case study to guide conservation strategies. These strategies will be based on the understanding of **i)** how the physical and chemical conditions of a headwater stream are modulated by seasonality, damming and by different precipitation regimes, and how these factors interact between them and with metal pollution, **ii)** how the biofilm and macroinvertebrate communities of the stream respond to those factors, and their role as bioindicators, **iii)** the differential use of the macroinvertebrate community as food resources by vertebrate predators in this changing context, and **iv)** the impacts of damming and metal pollution on the population of the Pyrenean brook newt *Calotriton asper* in the stream and the prevalence of the pathogenic fungi *Chytridium sp.* on it. To achieve these objectives, an initial two-years sampling was performed seasonally to characterize the abiotic parameters of the stream as well as the biofilm and macroinvertebrate communities, followed by additional samplings with specific objectives like the assessment of the diet of the vertebrate predators and the fish and newt populations.

These investigations are explained in 4 chapters of this PhD dissertation, with the following specific objectives:

- To analyse how the ecosystem structure and function of a Pyrenean headwater stream is driven by seasonality and affected by the interaction of damming, metal pollution and a precipitation decrease promoted by climate change in a conservation context that assesses their management options and future perspectives.

- **Chapter 1:** to assess how the physical and chemical parameters of the water and the metal pollution of a Pyrenean headwater stream are driven by seasonality, the presence of a hydroelectric dam, and different precipitation regimes, and how these factors modulate the ecosystem structure of the stream measured using biofilm and macroinvertebrate communities.

- **Chapter 2:** to characterize the macroinvertebrate community of the stream and assess its role as bioindicator of multiple ecosystem functions, using a functional feeding groups approach.
- **Chapter 3:** to study the prey electivity of the mammals *Galemys pyrenaicus* and *Neomys fodiens* and the bird *Cinclus cinclus* and the potential competition between them from the analyses of their diets. This information will be used to determine the trophic ecology of *G. pyrenaicus* and its diet specialization in order to assess how anthropogenic stressors could impair the access to proper food resources and habitats for this species, which is in serious danger of extinction.
- **Chapter 4:** to characterize the Pyrenean brook newt (*Calotriton asper*) population of the stream and assess the impacts of damming and metal pollution on it, considering its conservation implications.

The following hypotheses have been formulated:

i) Seasonality is the main driver of the water flow and physiochemical parameters of this Pyrenean headwater stream and, consequently, of its ecosystem structure and function.

ii) The water flow reduction that damming and a reduction of precipitations cause increases the stream temperature and promotes its autotrophy, which are detrimental for its biodiversity and the functioning of the ecosystem.

iii) Damming and a reduction of precipitations have a synergistic impact on the ecosystem structure and function of a headwater stream.

iv) Metal pollution in a headwater stream is detrimental for its ecosystem structure and function, and its effects are modulated by seasonality, damming and the precipitation regime.

v) Macroinvertebrates play a key role as consumers of basal resources and as prey for higher trophic levels. Consequently, the structure of its community has important implications for the conservation of higher trophic levels of the fluvial food web.

vi) In a situation of trophic competition, *Galemys pyrenaicus* is a specialized predator of energetically profitable aquatic, benthic macroinvertebrates.

vii) The population density and body size of the Pyrenean brook newt, *Calotriton asper*, is negatively affected by metal pollution.

viii) Ecosystem structure and function measures provide complementary, not redundant information about the effect of anthropogenic stressors on headwater streams.

ix) The use of multiple bioindicators provide different types of information about the ecological integrity of headwater streams.

x) Macroinvertebrates assessed as functional feeding groups can provide both a structural and a functional assessment of the ecological integrity of a headwater stream.

Materials and Methods

In this section of Materials and Methods, the fieldwork samplings, the laboratory analyses and the statistical treatments of the data performed on this thesis are indicated. All four chapters of this thesis implied samplings of different biological groups in a headwater stream from the Pyrenees. Samplings and different methodologies were performed in collaboration with other coauthors specified in each chapter. The methodology used in this thesis is summarized in this section but described in more detail in each chapter.

1. Field samplings

1.1. Study site and seasonal samplings (*Chapters 1, 2, 3 and 4*)

Over two years, between spring 2018 and winter 2020, a seasonal sampling was performed on 4 sites of the Catllar stream, a Pyrenean headwater stream from the northeast of the Iberian Peninsula (Figure 1), with a basin located between 1200 and 1600 m.a.s.l. During these samplings, biofilm and macroinvertebrate samples were taken, as well as physical and chemical measurements of the stream water. Biofilms were obtained from scrapping 25cm^2 of the surface of random stones in the riverbed, and macroinvertebrates were sampled performing the protocols from the Catalan Water Agency (ACA, 2006). Specific details about each sampling can be found on the respective chapters of this thesis. The obtained results were used for all chapters in this thesis, either directly or indirectly as the context to understand other results.

This stream is located at Vilallonga del Ter, between the municipalities of Setcases and Tregurà, between the kilometric points 9 and 10 of the GIV-5264 road. Sampling points were selected considering a relatively equidistant separation representative of the altitudinal zonation of the stream (Table 1). These points were separated by a hydroelectric dam, which differentiates upstream from downstream sampling points. In addition, an old abandoned antimony mine leaking metals and metalloids to the nearby waters was located West of Upst. 1. All samplings were performed following the mos adequate safety and ethical requirements, specified in each chapter.

The hydroelectric dam deviated most of the upstream water to the hydroelectric power station, which caused downstream water to come mainly from the adjacent west stream (Figure 2). The Catllar stream is found in the margins of two protection figures: the Natura 2000 network and the Natural Park of the Ter and Freser headwaters (Figure 3). The limit for these areas is found just 100m downstream from the dam. Consequently, the upstream section of the Catllar stream is located in a protected area, but not the downstream section.

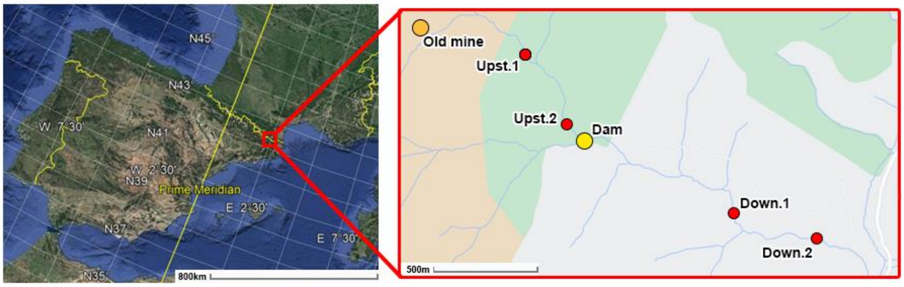

Figure 1. Catllar stream basin with its main tributaries, indicating the four sampling points with red dots (Upst 1, Upst 2, Down 1, and Down 2), the dam with a yellow dot, and the abandoned mine with an orange dot. Catllar stream is a tributary of the Ter River located in Catalonia, NE Iberian Peninsula (red square).

1.2. Fish community samplings (*Chapter 4*)

To characterize the fish community of the stream, electrofishing gear was used to sample a 100m-long transect at both Down. 1 and Upst. 2 sampling points using the IBICAT protocol, which consisted in a 100m-long transect, including all the stream width, was delimited with fishing nets and 3 successive catches were performed, removing all the fishes captured to avoid recaptures. (ACA, 2010).

Table 1. Sampling points on the Catllar stream with coordinates, relative position to the dam, and elevation above sea level.

Sampling point	UTM X	UTM Y	Position	Elevation
Upst. 1	439358	4691243	Upstream	1523m
Upst. 2	439676	4690703	Upstream	1427m
Down. 1	440749	4690214	Downstream	1217m
Down. 2	441681	4689735	Downstream	1172m

1.3. Habitat characterization (*Chapters 1* and *4*)

On ***Chapters 1*** and ***2***, the Fluvial Habitat Index (FHI) was assessed at each of the 4 sampling points in order to determine the hydromorphological characteristics of the river (Pardo et al., 2002; ACA, 2006).

1.4. Pyrenean newt samplings (*Chapter 4*)

The subpopulations of Pyrenean newt (*Calotriton asper*) at both sides of the dam were sampled visually at night, when they are the most active, along 500m-long transects at each side of the dam, including the 4 sites of seasonal samplings, using a two-event capture-recapture methodology (Chapman, 1951) to estimate their population density. Two samplings were performed on consecutive nights, considering the first sampling as capture, the second as recapture, and assessing which individuals from the second sampling were previously found on the first. Capture permits for *C. asper* were granted by the Catalan government and biosecurity measures were ensured during all the samplings.

On ***Chapter 4***, the sampled transects were characterized by identifying all the potential habitats and refugia for *Calotriton asper* following the characterization methodology proposed by Montori et al. (2008).

Figure 2. Hydroelectric dam of the Catllar stream. Upstream water is deviated to the hydroelectric power station. Downstream water comes from an adjacent stream that falls into an underground passage.

1.5. Insectivorous vertebrate diet samplings (*Chapter 3*)

Faeces and depositions of *Galemys pyrenaicus*, *Neomys fodiens* and *Cinclus cinclus* were obtained manually at both sides of the dam in order to assess their diet. The macroinvertebrates found in them were identified morphologically and counted to assess their prey electivity.

2. Main analytical methods

The main analytical methods are hereby summarized in the following sections (Table 2).

2.1. Atmospheric and precipitation data (*Chapters 1* and *2*)

Air temperature, precipitation and other atmospheric data were obtained daily from a meteorological station located in the Catllar basin between spring 2018 and winter 2020. Because precipitation regimes were different between seasons and years, the the average daily and accumulated precipitation data from the in situ meteorological station, plus the water flow data obtained from the Sant Joan de les Abadesses flow station were used to categorize each season as Wet (spring 2018, autumn 2018 and winter 2020) or Dry (spring 2019, autumn 2019 and winter 2019), in relation to their opposite one.

Figure 3. Catllar stream map showing the area protected by the Natura 2000 and the Natural Park legal figures upstream from the dam.

2.2. Physical and chemical analyses of the water (*Chapter 1*)

- Water temperature, dissolved oxygen, electrical conductivity and pH of the stream water using a WTW multiprobe.
- Water flow using the mass balance method (Hudson and Fraser, 2005).
- Water metal and metalloid concentrations using an ICP-MS.

2.3. Biofilm measurements (*Chapter 1*)

- Biofilm samples were obtained by scrapping 25 cm^2 of stones from the riverbed and stored in 30 ml vials with stream water.
- Benthic chlorophyll-a, extracted with 90% acetone from the biofilms, following the methodology of Jeffrey and Humphrey (1975), and determined using a spectrophotometer Shimadzu UV-1800.
- Ash free dry mass (AFDM) of the biofilms (dried, dehydrated and weighted).
- Algal and diatom community identification to a genera level using a light microscope Nikon E600 (Tokyo, Japan) with Nomarski differential interference contrast optics.
- Algal cell biovolume, using a Nikon E600 light microscope, following a set of geometrical shapes proposed by Hillebrand et al. (1999).
- Biofilm metal and metalloid concentrations using an ICP-MS. Samples were previously lyophilized and microwave-digested.

2.4. Macroinvertebrate measurements (*Chapters 1, 2 and 3*)

Chapters 1, 2 <u>and</u> ***3***

- Macroinvertebrate samplings based on the quantitative protocols from the Water Catalan Agency (ACA, 2006) and the Spanish Ministry of Agriculture, Alimentation and Environment (MAGRAMA, 2013).
- Macroinvertebrate identification in diet samples using a stereoscopic light microscope Optika SZR-10 and identification keys (Campaioli, 1999; Malicky, 2004; Tachet et al., 2010; Oscoz et al., 2011).
- Dry mass using a Raypa DOD-20 oven and a Satorius Practum 124-1S analytical scale.

- Biodiversity indices calculated: IBMWP (Alba-Tercedor et al., 2002), IASPT (Armitage et al., 1983) and EPT (Lenat, 1988).

<u>Chapter 2</u>

- Functional feeding groups assigned using the classification proposed by Tachet et al. (2000).

2.5. Body size measurements (*Chapter 4*)

- Fish individuals (*Salmo trutta fario*) were weighted and measured from the tip of the snout to the posterior end of the last vertebra (standard length).
- Pyrenean newts (*Calotriton asper*) measured using the total length (TL), snout-to-vent length (SVL), weighted, and sexed. Ventral part of the individuals photographed to identify them using the software Interactive Individual Identification System (I3S Pattern).

2.6. Genetic analyses (*Chapter 4*)

- Pyrenean newts (*Calotriton asper*) swabbed to obtain DNA samples in order to do a PCR to check for *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* presence in the population.

2.7. Diet analyses

- Macroinvertebrate identification in diet samples of *Neomys fodiens, Galemys pyrenaicus* and *Cinclus cinclus*, using a stereoscopic light microscope Optika SZR-10 and identification keys (Campaioli, 1999; Malicky, 2004; Tachet et al., 2010; Oscoz et al., 2011).

Table 2. Summary of the different analytical methods used for atmospheric, water and biological (biofilm, macroinvertebrates and vertebrate animals) data in each chapter (***Ch.***) of this thesis.

	Ch. 1	***Ch. 2***	***Ch. 3***	***Ch.4***
Atmosphere				
Precipitation	🟪	🟪		
Water				
Physical and chemical parameters	🟦	🟦		🟦
Water flow	🟦	🟦		🟦
Metal and metalloid concentration	🟦	🟦		🟦
Biofilm				
Chlorophyll-*a*	🟩	🟩		
Ash free dry mass	🟩	🟩		
Algal community	🟩	🟩		
Biovolume	🟩	🟩		
Metal and metalloid concentration	🟩	🟩		🟩
Macroinvertebrates				
Community assemblage	🟧	🟧	🟧	
Biodiversity indices	🟧	🟧		
Functional feeding groups		🟧		
Vertebrate animals				
Fish weight and length				🟨
Newt weight and length				🟨
Chytridiomycosis PCR				🟨
Diet samples			🟨	

2.8. Statistical analyses

Statistical analyses performed in this thesis are summarized in Table 3.

Table 3. Summary of the different statistical methods used in each chapter (**Ch.**) of this thesis.

Statistical analysis	Purpose of the analysis	Ch. 1	Ch. 2	Ch. 3	Ch.4
Box-Cox transformation	Used to reach data normality and homoscedasticity when other data transformations were not useful	■	■		
Analysis of Variance (ANOVA) applied to a Linear Mixed-Effects model (LME)	To determine differences in the analysed physical, chemical, biofilm and macroinvertebrate parameters between seasons, positions from the dam and precipitation regimes	■	■		
Analysis of Similarities (ANOSIM)	To assess differences in the biological biofilm and macroinvertebrate community between seasons, positions and precipitation regimes	■	■		
Principal Components Analysis (PCA)	To assess differences in the biofilm and macroinvertebrate community within and between years	■	■		
Functional feeding group ratios	To estimate ecosystem functional parameters using the abundances of different macroinvertebrate functional feeding groups		■		
Pianka niche overlap index	To determine the degree of trophic overlap between *G. pyrenaicus, N. fodiens* and *C. cinclus*.			■	
Ivlev's electivity index	To assess the macroinvertebrate prey electivity considering their availability in the environment by the three analysed vertebrate predators			■	
T-Student	To compare weight and length measures of *Calotriton asper* subpopulations between positions from the dam				■
Analysis of Covariance (ANCOVA)	To assess the differences in the physiological condition of the subpopulations of *C. asper* and *S. trutta fario* between positions from the dam				■
Chapman estimation	To estimate the population density of *Calotriton asper*				■
Carle-Strub estimation	To estimate the population density of *Salmo trutta fario* in the stream				■
Chi-Square test	To compare fish sizes between positions from the dam				■

Results

Chapter 1

Impacts of damming and climate change on the ecosystem structure of headwater streams: a case study from the Pyrenees

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1. Introduction

Freshwater biodiversity is highly threatened by many human-mediated processes and stressors like pollution, habitat destruction, flow modification, over-extraction of water, overfishing, alien species introduction and climate change (Revenga et al., 2005; Dudgeon et al., 2006; Dudgeon, 2010; Ormerod et al., 2010). Therefore, rivers and streams are currently among the most exploited ecosystems in the planet (Loh et al., 2005). These threats can cause changes in the composition of the biotic community of a stream, usually with a simplification and impoverishment of its biodiversity and other structural components (Maddock, 1999), reducing the ability of the stream to recover from disturbances and stressors and affecting ecosystem functioning (Bona et al., 2008; Sabater and Tockner, 2009). If we aim to conserve, manage and, if needed, restore streams, while at the same time assuring goods, services and energy to human populations, we need to assess how these threats can compromise the structure and function of these ecosystems (Freeman et al., 2007).

One of the main threats to the conservation of stream ecosystems is the construction of dams and weirs as renewable energy sources, which is being promoted in order to reduce greenhouse gas emissions and to supply electricity to more than 940 million people, especially in low-income regions (The World Bank, 2018; Zarfl et al., 2019). Despite their social benefits, dams have many potential serious and long-term downsides for the ecosystems: habitat degradation and fragmentation, biodiversity loss, ecosystem services erosion, changes in hydrology and sediment transport, flow reduction, water temperature increase and deterioration of water quality, among others (Bednarek, 2001; Lessard and Hayes, 2003; Zarfl et al., 2019). Water flow is considered a master variable on stream ecosystems as it structures the habitat, influences physical and chemical parameters, and controls population and community dynamics. Because stream communities are adapted to a natural water flow variability, its alteration caused by damming can impact the structure and function of these ecosystems (Bednarek, 2001; Mor et al., 2018).

Since the 20th century, the hydromorphological alteration of streams has been coupled with the proliferation of industries and mining, causing an increase in the metal and metalloid concentrations of their waters and sediments (Colas et al., 2013). These pollutants can bioaccumulate in the organisms, transforming into persistent metallic compounds with high toxicity

(Jin, 1992), but they can also accumulate in the sediments retained by a dam, impairing the local biological communities and threatening distant downstream communities due to flush-outs or dam removals (Colas et al., 2013). Current research on metal pollution is integrating ecological principles on its design and implementation in order to properly assess the impacts of metals and metalloids on ecosystems and biological communities (Gessner and Tlili, 2016). Therefore, evaluating the metal pollution situation in streams affected by damming can provide a better understanding of how metals and metalloids can impact biological communities in hydrologically altered streams.

Current pressures on stream ecosystems will be exacerbated by the effects of climate change, severely impacting their ecosystem structure and function (Walther et al., 2002; Parmesan and Yohe, 2003; Sabater and Tockner, 2009; Muñoz-Mas et al., 2018). Headwater streams are a type of lotic ecosystem that are especially vulnerable to climate change. They are first- and second-order streams characterized by low water temperatures, distinct seasonal and daily flow variations, oligosalinity and singular hydrological and morphological conditions (Bona et al., 2008). The ecosystem structure of headwater streams is driven by a marked seasonal flow variability (Scarsbrook and Townsend, 1993; Biggs et al., 2005), as the hydrological regime on these systems is regulated by processes of snow accumulation and snowmelt that drive low and uniform winter flows, and highwater flows during the snowmelt in spring and early summer (López-Moreno and Beniston, 2009a; Sanmiguel-Vallelado et al., 2017). Climate change is expected to alter this seasonal pattern due to a decrease in the precipitation regime and in the snow cover (Cramer et al., 2018). Because freshwater organisms in headwater streams are adapted to a cold and natural flow (Poff et al., 1997), these alterations caused by climate change can lead to strong impacts on the ecosystem structure of headwater streams (Perkins et al., 2010). Climatic models of the Pyrenees, a mountain range located in North-West Iberian Peninsula (Western part of the Mediterranean Basin, predict, by the end of the 21st century, a decrease in precipitations of between 10.7 and 14.8% and a mean increase in temperatures of between 2.8 and 4 °C (López-Moreno et al., 2008a), as well as a decrease in the maximum level of accumulated snow (López-Moreno et al., 2009a). Therefore, this mountain range is a suitable area to research how climate change can impact the ecosystem structure of headwater streams.

Collectively, these previously mentioned anthropogenic threats can cause stress on the biotic communities of headwater stream ecosystems. Ecosystem stress is defined as a condition caused by environmental factors that bring organisms near the edges of the reference ranges for a determined ecological function. These stressors cause communities to adapt their ecological niches to new environmental conditions (Straalen, 2003; Steinberg, 2012). Multiple natural and anthropogenic stressors can occur simultaneously, causing complex responses by the biotic communities. These can be additive if the effects of multiple stressors equal the sum of their individual effects, or multiplicative if the resultant effect is greater (synergism) or lesser (antagonism) than the sum of their individual effects (Piggott et al., 2015; Jackson et al., 2016). In this regard, the impacts of climate change on the ecosystem structure of headwater streams could be exacerbated by the flow reduction that damming implies. Furthermore, both climate change and damming could interact with the metal pollution situation that many stream ecosystems experience, as the flow reduction could increase their concentration.

Understanding how these multiple anthropogenic stressors interact to impair the ecosystem structure of headwater streams is key for the effective management and conservation of these ecosystems. In this context, this research paper presents a case study of a Pyrenean headwater stream affected by the presence of a hydroelectric dam and by metal pollution from an old antimony mine, in a climate change scenario. The main focus of this case study is to assess the response of the ecosystem structure, in a seasonal context, to the presence of a dam, a reduction in precipitation, and to metal pollution. We hypothesized that damming and the reduction in precipitations will decrease the water flow of the stream and increase its temperature, affecting other abiotic parameters and the concentration of metal and metalloid pollutants, which will have negative consequences for the ecosystem structure of the stream. Even more, we expect these stressors to interact between, modulated by seasonal patterns, having multiplicative synergic effects on this ecosystem structure.

2. Materials and methods

2.1. Study area and data collection

The study area is a small Pyrenean headwater stream named Catllar, an influent of the Ter River, in Vilallonga del Ter, Catalonia (NE Iberian Peninsula), located between 1200 and 1600 m.a.s.l. We selected four sampling points (Upst 1, Upst 2, Down 1 and Down 2) considering a similar separation between them, representative of the altitudinal zonation of the stream. A hydroelectric dam located between the Upst 2 and Down 1 sampling points divided the stream. It deviated most of the upstream water to a hydroelectric station, and downstream water comes mainly from the adjacent west stream. An abandoned Sb mine was present west to the Upst 1 sampling point (General materials and methods: Figure 1), which was a source of metals and metalloids in the stream. We took water samples and physical and chemical parameter measures, and sampled biofilms and macroinvertebrates at each sampling point each season during two years between spring 2018 and winter 2020, with the exception of summer 2018, resulting in seven samplings per point.

2.2. Biofilm samplings and analyses

Biofilm samples were taken by scrapping 25 cm^2 of the surface of random stones in the riverbed and stored in 30 ml vials with river water. Four repetitions for each sampling were performed in order to obtain samples for the algal community characterisation, Chlorophyll-*a* (Chl-*a*) concentration (algal biomass), total biomass expressed as Ash Free Dry Mass (AFDM) and metal and metalloid bioaccumulation. Chlorophyll-a and AFDM samples were frozen until analysis. For the algal community analysis, a 15 µl drop of 4% formalin was added to the fresh sample to preserve it and the sample was chilled until its analysis. The metal bioaccumulation samples were frozen, lyophilized and then microwave-digested using vials with nitric acid and hydrogen peroxide inside Teflon pressure vessels with water and hydrogen peroxide. The metal concentrations in the digested biofilm samples were then measured in an Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS). To analyse the Chl-*a* concentration and AFDM of the biofilm samples, we used 47 mm diameter glass-fibre filter papers to void-filter them. The solid residue was put in a glass container with 20 ml of 90% acetone to extract its Chl-*a*, and was preserved in cold, covered from light, for 24 hours. After that period, it was sonicated (J.P. Selecta, Barcelona) for two minutes to promote cell lysis and improve the Chl-*a* extraction and then, using another pre-dried and weighted filter paper, it was void-filtered again. Extra acetone was used to wash all the biofilm, and the total volume used was considered for the calculations. The filtered acetone contained the Chl-*a* of the sample, which was measured using a spectrophotometer (Shimadzu UV-1800) following the methodology described by Jeffrey and Humphrey (1975). The residue on the paper was dried at 50 °C over a week until constant weight, using a drying oven (Raypa DOD-20), and weighted it using an analytical scale (Sartorius Practum 124-1S) in order to measure the dry mass of the biofilm. Afterwards, it was dehydrated at 450 °C for four hours and weighted again to measure its AFDM. These values were used to calculate the percentage of organic matter (proportion of dry mass weight from the total weight) and the autotrophic index (AFDM divided by the Chl-*a* concentration) of the samples. In order to identify the algal community composition of the biofilms, a 15 µl drop of the scrapped samples was mounted under a 22 mm x 22 mm slide cover and observed with an optic microscope (Nikon E600, Tokyo, Japan) at 400x to identify algae (mainly diatoms) to a genera taxonomic level, counting until 400 cells. Twenty-five cells per genera were measured in order to estimate its biovolume from the shape equations proposed by Hillebrand et al. (1999). Total algal biovolume per sample was calculated by multiplying the cell abundance by the calculated biovolume for each genus, and then expressed as density (µm^3/cm^2) considering all the surface and volume measurements. Shannon diversity index using the genera count was also calculated.

2.3. Macroinvertebrate samplings and analyses

Macroinvertebrate samplings were based on the quantitative macroinvertebrate sampling protocols from the Catalan Water Agency (ACA, 2006) and the Spanish Ministry of Agriculture, Food and Environment (MAGRAMA, 2013). For each sampling point, a coverage percent estimation for each type of microhabitat substrate was assessed. Ten sampling units covering 0,125 m^2, using a 25 cm wide and 500 µm mesh size hand net, were taken at each point proportionally distributed for each microhabitat type: one sampling unit per each 10% of microhabitat coverture, plus an extra half sampling unit for really minority habitats (<5%). Samples were stored in plastic containers with ethanol 80% until their processing and analysis at the laboratory. Macroinvertebrates were identified using a stereoscopic microscope (Optika SZR-10) by means of identification keys (Campaioli, 1999; Malicky, 2004; Tachet et al., 2010; Oscoz et al., 2011). Taxonomic resolution was based on the IBMWP (Iberian Biological Monitoring Working Party) Biological Quality Index (Alba-Tercedor et al., 2002) in which taxa were identified up to family level, except for Nematoda, Nematomorpha, Oligochaeta, Ostracoda, Hydracarina and Collembola. For each sample, a fraction of at least 300 individuals was identified and counted. All the individuals of the taxa that were not present in the analysed fraction, but were found in the rest of the sample, were also counted. Abundance for each taxon was calculated by extrapolating the abundance of all individuals present in the analysed fraction plus the number of individuals in the rest of the sample. Considering the sampled area, results were expressed as density (individuals/m^2). The dry mass of each taxon in the samples was assessed using a representative sub-sample of each taxon dried at 60°C, until constant weight, using a drying oven (Raypa DOD-20) and weighted using an analytical scale (Sartorius Practum 124-1S). For biomass calculations, sub-samples that did not reach the detection limit (DL = 1 mg) had a value assigned of 0.7·DL. Samples that had 30% of the taxa under the detection limit were not considered (Bennet et al., 2000). Total weight of each sub-sample was divided by their total number of individuals. These values were multiplied by the abundance of each taxon and expressed as mg of dry mass/m^2. Finally, three Biological Quality Indexes for macroinvertebrates were calculated: IBMWP (Alba-Tercedor et al., 2002), IASPT (Iberian Average Score per Taxon) (Armitage et al., 1983), and EPT (Ephemeroptera, Plecoptera and Trichoptera Index) (Lenat, 1988).

2.4. Physical and chemical parameters

We measured water temperature, conductivity, pH and oxygen concentration at each sampling point by using a multiprobe (WTW), and the water flow by using the mass balance method (Hudson and Fraser, 2005). We also analysed the metal (Cr, Ni, Cu, Zn, and Cd) and metalloid (As and Sb) concentrations in the water by filtering 5 ml of stream water through nylon fibre filters, adding 15 µl of nitric acid to the sample in order to avoid metal precipitation, and then detecting them by using an ICP-MS. Hydromorphological characteristics of the stream were assessed at each sampling point by applying the Fluvial Habitat Index (FHI) (Pardo et al., 2002; ACA, 2006). Air temperature, precipitation, wind speed and atmospheric pressure data were obtained in-situ from a meteorological station, which measured these parameters on a daily basis from spring 2018 to winter 2020. Additionally, data from the water flow of the Ter River were obtained in the nearest water flow station at the locality of Sant Joan de les Abadesses, which was used to obtain the monthly average flow between 2009 and 2020 in order to understand the seasonality of the water flow in this Pyrenean area.

2.4. Statistical analyses

Statistical analyses were performed using the RStudio software version 1.2.5033 (RStudio Team, 2019). Due to the lack of the summer 2018 sampling, and consequently the lack of a replicate for this season, we removed the summer data from the statistical analyses, considering summer 2019 information only as descriptive. Biofilm, macroinvertebrate, physical and chemical variables were checked and, if data did not meet normality and homoscedasticity assumptions, it was transformed using logarithms, square roots or Box-Cox transformations (Box and Cox, 1964) as best suited. Precipitation regimes were different between seasons and years, so in order to assess the potential effects of climate change, we used the average daily and accumulated precipitation data results from an in situ meteorological station, plus the water flow data from the Sant Joan de les Abadesses flow station to categorize each season as Wet (spring 2018, autumn 2018 and winter 2020) or Dry (spring 2019, autumn 2019 and winter 2019), in relation to their opposite one. To account for the influence of seasonality and assess the impact of damming and the different precipitation regimes on the abiotic and biotic structural parameters of the Catllar stream, variables were grouped by season (Spring, Autumn and Winter), relative position from the dam (Upst. 1 and Upst. 2= Upstream, Down. 1 and Down. 2= Downstream), and precipitation regime (Dry or Wet). We then applied a Linear Mixed-Effects model (LME) for each variable by considering the season, the relative position from the dam and the precipitation regime as fixed effects, and the sampling point (Upst. 1, Upst. 2, Down. 1 and Down. 2) as the random effect.

In order to assess the differences in the biofilm and macroinvertebrate community composition between seasons, relative position from the dam and precipitation regime, we performed an Analysis of Similarities (ANOSIM) using the vegan R package (Oksanen et al., 2019). Algal genera biovolumes and macroinvertebrate family biomasses, excluding those representing less than 1% of the total biomass, were used as dependent variables. In addition, we also performed a Principal Component Analyses (PCA) of the biofilm and macroinvertebrate data for each sampling with the objective of observing differences in their community composition within and between years.

3. Results

3.1. Seasonality

Cold averaged daily temperatures were observed all year long, with a maximum of 17 °C in summer 2019 and a minimum of 5.3 °C in winter 2019. Precipitation regimes were different between seasons and years, with a maximum of 546.2 mm of accumulated rain in autumn 2018, and a minimum of 44.6 mm in winter 2019. This Pyrenean precipitation variability was reflected in the San Joan de les Abadesses flow station with a high water flow variability, caused by high or low precipitation regimes. From 2016 to 2020, the water flow was highly variable seasonally and interannually (General Introduction: Figure 4), allowing us to compare each season as wet or dry during the two years of this study (2018-2020). In the Catllar stream, this strong seasonal pattern was reflected in the water flow and temperature variability. Water temperature, conductivity, oxygen concentration, pH and flow differed between seasons (Table 1 and Table 2). Metal and metalloid concentrations of Cr, Zn, As, Cd and Sb in the stream water, as well as the concentration of Cr, Zn and Cd in biofilms, also differed between seasons (Table 3 and Table 4).

The ANOSIM analysis showed that the community composition of algal genera in the biofilms (n=16) and of macroinvertebrate families (n=23) was significantly different between seasons (algal genera: R=0.217, p=0.004; macroinvertebrate families: R= 0.519, p=0.001), but not between the relative position to the dam nor between precipitation regimes. The PCA analysis showed that, for the algal community, the highest diversity was found during winter (Figure 1). In the dry winter of 2019, we observed a dominance of the diatom genera *Fragilaria, Diatoma, Cyclotella* and *Cymbella*, which were dominant also during the dry spring and summer of 2019. In the wet winter of 2020, we predominantly found *Hydrurus, Amphora, Cocconeis* and *Oscillatoria*. It was remarkable that, during winter, a bloom of the macroalgae *Hydrurus foetidus* (Villars) Trevisan 1848 was observed in the riverbed. For the macroinvertebrate community composition (Figure 2), most of the diversity was found in winter and spring, with most of the families being shared between both seasons.

Considering the ecosystem structure parameters, the AFDM and Chl-*a* concentration in biofilms was the highest during winter, coinciding with the macroinvertebrate abundance peak (Table 1 and Table 2). It was observed that

Table 1. Average and standard deviation values of multiple water, biofilm and macroinvertebrate variables analysed grouped by season, relative position to the dam and precipitation regime. The number of samples indicate how many seasonal replicates were used for the calculations. Each seasonal replicate includes the averaged values of the four sampling points along the stream.

Parameter	*Season*				*Position*		*Precipitation regime*	
	Spring	Summer	Autumn	Winter	Upstream	Downstream	Wet	Dry
Water								
Temperature (°C)	7.6 ± 1.4	15.1 ± 1.1	4.4 ± 1.1	5.7 ± 0.8	5.2 ± 1.6	6.6 ± 1.6	5.9 ± 1.5	6.0 ± 1.9
Conductivity (µS/cm)	153 ± 3.9	192 ± 26.1	161 ± 29	157 ± 37.8	128 ± 14.2	186 ± 18.2	145.8 ± 28.6	168.8 ± 33.4
Oxygen (mg/L)	9.5 ± 0.3	8.1 ± 0.15	10.5 ± 0.2	10.1 ± 0.1	10.1 ± 0.4	9.9 ± 0.4	10.0 ± 0.4	10.0 ± 0.5
pH	8.1 ± 0.2	8.0 ± 0.04	7.9 ± 0.2	7.8 ± 0.2	7.9 ± 0.2	8.0 ± 0.2	8.0 ± 0.2	7.9 ± 0.2
Flow (m$^{3/}$s)	0.2 ± 0.1	0.07 ± 0.01	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
FHI	69 ± 4.2	74 ± 1.0	66 ± 2.1	73 ± 4.0	68.2 ± 4.1	70.7 ± 4.5	69.6 ± 3.7	69.3 ± 5.2
Biofilms								
Algal biovolume (μm^3/cm)	1.5x10^8 ± 8.1x10^7	8.4x10^7 ± 1.1x10^8	3.8x10^8 ± 2.5x10^8	7.1 x10^8 ± 5.4x10^8	3.4x10^8 ± 2.4x10^8	4.8x10^8 ± 5.3x10^8	4.8x10^8 ± 5.4x10^8	3.4x10^8 ± 2.1x10^8
Biomass (µg Chl-a/cm^2)	18.1 ± 4.7	28.4 ± 13.2	29.0 ± 14.8	182 ± 109	73.2 ± 99.7	80.0 ± 97.5	57.6 ± 42.3	95.6 ± 130
AFDM (mg/m^2)	2297 ± 1756	464 ± 175	653 ± 215	6204 ± 4596	2912 ± 3526	3190 ± 3812	2454 ± 2701	3648 ± 4358
Algal Shannon diversity	1.9 ± 0.4	1.3 ± 0.5	2.1 ± 0.2	2.1 ± 0.4	2.1 ± 0.2	1.9 ± 0.4	2.1 ± 0.3	2.0 ± 0.4
Algal genera richness	7.8 ± 2.0	4.7 ± 1.9	11.0 ± 1.2	10.0 ± 1.7	10.0 ± 1.3	9.7 ± 2.7	10.2 ± 2.0	9.0 ± 2.1
Autotrophic Index	127 ± 81.2	16.8 ± 2.9	161 ± 29.8	157 ± 37.8	128 ± 14.2	186 ± 18.2	145 ± 28.6	168 ± 33.4
% Organic matter	34.1 ± 7.7	43.3 ± 9.6	39.4 ± 19.4	54.4 ± 33.3	33.7 ± 21.4	51.5 ± 23.7	38.4 ± 20.3	46.9 ± 27.0
Macroinvertebrates								
Biomass (mg/m^2)	2931 ± 768	2270 ± 665	1257 ± 201	2839 ± 811	2162 ± 944	2523 ± 1040	2331 ± 870	2354 ± 1133
Abundance (ind/m^2)	3273 ± 335	3342 ± 995	3317 ± 598	4206 ± 956	3716 ± 787	3481 ± 809	3272 ± 543	3925 ± 891
Families	37.2 ± 1.7	37 ± 2.7	32.1 ± 1.6	33.1 ± 3.1	34.0 ± 3.8	34.3 ± 2.3	35.3 ± 3.3	33.1 ± 2.7
IBMWP	234 ± 6.8	223 ± 12.8	206 ± 9.2	212 ± 14.6	216 ± 18.1	218.8 ± 13.2	221± 16.6	214 ± 14.2
IASPT	6.3 ± 0.2	6.1 ± 0.1	6.4 ± 0.2	6.4 ± 0.2	6.4 ± 0.3	6.4 ± 0.1	6.3 ± 0.2	6.5 ± 0.2
EPT	17.9 ± 0.6	16.3 ± 0.8	16.6 ± 0.9	16.6 ± 1.3	16.7 ± 1.3	17.4 ± 0.8	17.2 ± 1.0	16.9 ± 1.3
Number of samples	2	1	2	2	6	6	6	6

Table 2. Results of the Linear Mixed-Effects Model (LME) for multiple water, biofilm and macroinvertebrate variables analysed: effects of season, relative position to the dam and precipitation regime (P.R.). The degrees of freedom are 1 for position, precipitation regime and their interaction, and 2 for season and its interactions with position and precipitation regime. Variables were transformed using $\log_{10}$, square root or Box-Cox to reach normality. Significant results ($P<0.05$) are in boldface.

Parameter	Season		Position		P.R.		Season*Position		Season*P.R.		Position*P.R.		Season*Position*P.R.	
	χ2	*P*	χ2	*P*	χ2	*P*	χ2	*P*	χ2	*P*	χ2	*P*	χ2	*P*
Water														
Temperature	185.93	**<0.05**	3.52	0.06	0.19	0.66	10.03	**<0.05**	16.11	**<0.05**	4.60	**<0.05**	4.29	0.12
Conductivity	204.46	**<0.05**	386.79	**<0.05**	2539	**<0.05**	4.11	0.13	2072	**<0.05**	175.37	**<0.05**	3.50	0.17
Oxygen	272.10	**<0.05**	2.36	0.12	0.12	0.73	4.08	0.13	7.24	**<0.05**	6.72	**<0.05**	2.22	0.33
pH	31.32	**<0.05**	4.93	**<0.05**	3.40	0.06	0.28	0.87	31.71	**<0.05**	0.03	0.86	2.11	0.35
Flow	10.38	**<0.05**	7.10	**<0.05**	112.46	**<0.05**	5.17	0.07	6.43	**<0.05**	0.46	0.50	5.37	0.07
FHI	19.58	**<0.05**	3.98	**<0.05**	0.07	0.79	1.38	0.50	8.74	**<0.05**	4.53	**<0.05**	1.73	0.42
Biofilms														
Algal biovolume	22.45	**<0.05**	0.94	0.33	0.41	0.52	4.46	0.12	7.55	**<0.05**	2.94	0.09	3.29	0.19
Biomass	231.24	**<0.05**	0.02	0.89	19.11	**<0.05**	1.50	0.47	30.81	**<0.05**	0.52	0.47	7.73	**<0.05**
AFDM	28.15	**<0.05**	0.08	0.78	0.44	0.51	7.38	**<0.05**	13.21	**<0.05**	3.35	0.06	1.80	0.41
Algal Shannon diversity	0.92	0.63	1.40	0.23	0.18	0.67	5.80	**0.05**	0.91	0.63	1.32	0.25	1.13	0.57
Algal genera	22.45	**<0.05**	0.94	0.33	0.41	0.52	4.46	0.11	7.55	**<0.05**	2.94	0.09	3.29	0.19
Autotrophic Index	37.09	**<0.05**	0.01	0.93	2.52	0.11	10.21	**<0.05**	6.75	**<0.05**	6.81	**<0.05**	4.62	0.10
% Organic matter	0.79	0.67	4.95	**<0.05**	1.06	0.30	2.70	0.26	1.89	0.39	2.57	0.11	2.39	0.30
Macroinvertebrates														
Biomass	108.20	**<0.05**	4.71	**<0.05**	0.37	0.54	1.96	0.37	21.70	**<0.05**	5.25	**<0.05**	1.84	0.40
Abundance	13.44	**<0.05**	0.34	0.56	7.76	**<0.05**	0.34	0.84	6.08	**<0.05**	0.10	0.76	2.75	0.25
Families	45.71	**<0.05**	0.26	0.61	10.90	**<0.05**	6.42	**<0.05**	16.48	**<0.05**	0.06	0.78	1.58	0.45
IBMWP	42.27	**<0.05**	0.28	0.59	4.21	**<0.05**	1.86	0.39	14.80	**<0.05**	0.51	0.47	1.42	0.49
IASPT	4.14	0.13	0.16	0.69	7.22	**<0.05**	7.91	**<0.05**	2.75	0.25	2.75	0.10	0.46	0.79
EPT	13.46	**<0.05**	4.16	**<0.05**	0.44	0.09	4.79	0.09	10.83	**<0.05**	1.06	0.30	0.86	0.65

water temperature was negatively correlated with algal genera richness in biofilms (Figure 3). The highest number of macroinvertebrate families and values of the IBMWP and EPT quality indices were observed during spring (Table 1).

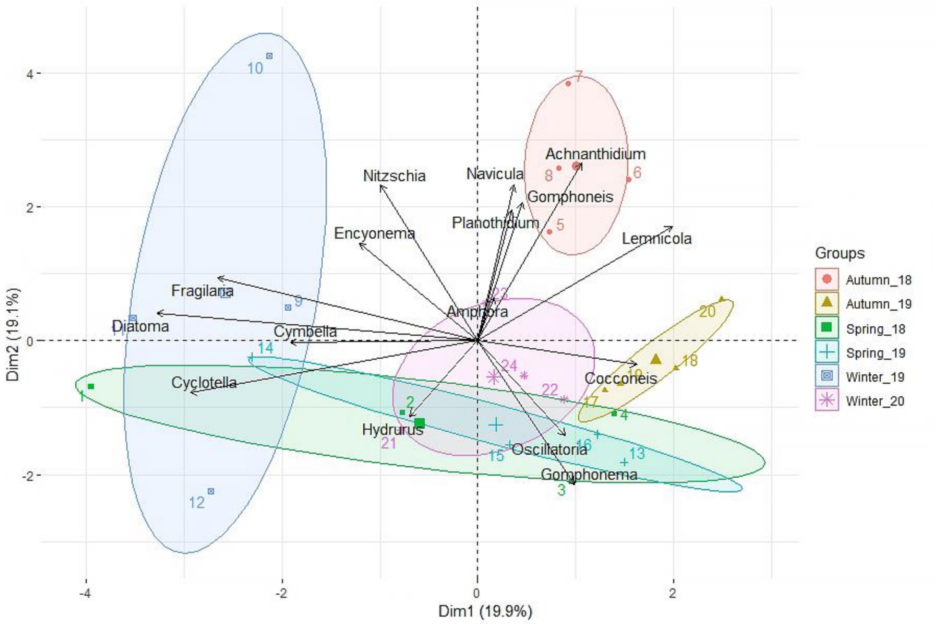

Figure 1. PCA plot showing community differences between seasons for algal genera (expressed in biovolume) found in biofilms. Macroalgae *Hydrurus foetidus* was found in the streambed during cold seasons.

3.2. Effects of damming and its interaction with the seasonal responses of the ecosystem

Damming altered the physical and chemical characteristics of the downstream section of the stream, by reducing the water flow and increasing the water temperature, conductivity, pH and the FHI (Table 1 and Table 2). Water temperature was higher downstream than upstream during all the seasons. Metal and metalloid concentrations were also affected by damming, as the concentration in biofilms of Ni, Cu and As, as well as the As concentration in water, were significantly lower in the downstream section of the stream (Table 3 and Table 4). The presence of the dam by itself also impacted the ecosystem structure of the stream, as it increased downstream the relative organic matter content of the biofilms, the macroinvertebrate biomass and the EPT index (Table 1 and Table 2). The seasonal pattern of the ecosystem structure of the stream was altered by the presence of the dam. During cold seasons, downstream of the dam the AFDM and autotrophic index of the biofilm was higher and the algal and macroinvertebrate diversity was lower than upstream (Figure 4).

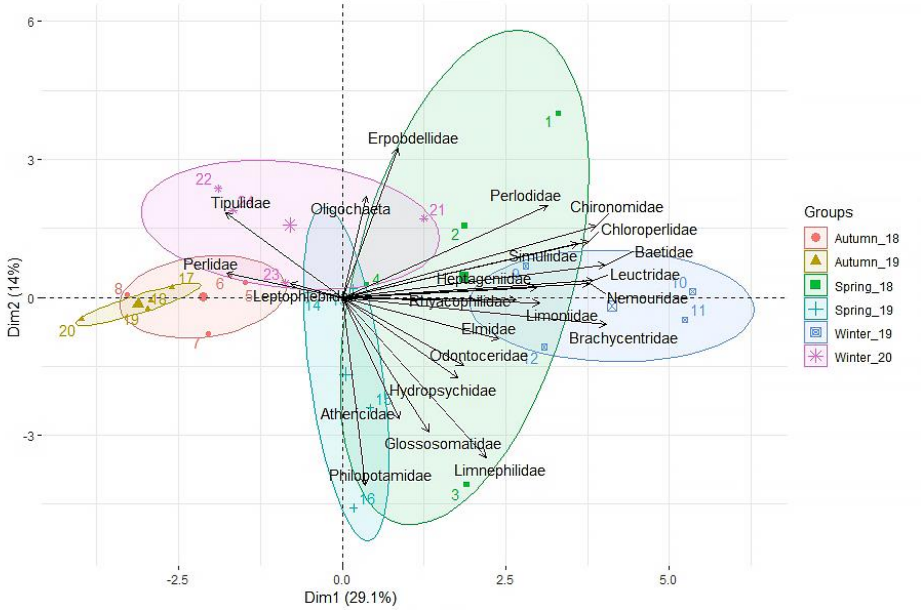

Figure 2. PCA plot showing community differences between seasons for macroinvertebrate families (expressed in biomass).

3.3. Effects of the precipitation regime and its interaction with the seasonal responses of the ecosystem

Different precipitation regimes significantly altered the physical and chemical characteristics of the stream by diminishing the water flow during low precipitation regimes, increasing its conductivity and the As concentrations in water and in biofilms. The ecosystem structure was also affected by the different precipitation regimes, as a significant increase of algal biomass (Chl-*a* concentration) and macroinvertebrate abundance, as well as a decrease in macroinvertebrate family richness, was observed during the dry regime. Macroinvertebrate quality indices also differed significantly depending on the precipitation regime, with the exception of EPT (Table 1 and Table 2).

Table 3. Average and standard deviation of metal (Cr, Ni, Cu, Zn, Cd) and metalloid (As, Sb) concentrations in water and in dry biofilm grouped by seasons, relative position to the dam and precipitation regime. The number of samples indicate how many seasonal replicates were used for the calculations. Each seasonal replicate includes the averaged values of the four sampling points along the stream.

Metals and metalloids	Season				Position		Precipitation regime	
	Spring	Summer	Autumn	Winter	Upstream	Downstream	Wet	Dry
Water (µg/L)								
Chromium	0.2 ± 0.1	2.8 ± 0.4	1.3 ± 0.3	1.0 ± 0.8	0.9 ± 0.9	0.8 ± 0.9	0.8 ± 0.8	0.9 ± 1.0
Nickel	0.5 ± 0.3	2.7 ± 1.8	0.8 ± 0.7	0.6 ± 0.5	0.5 ± 0.3	0.8 ± 0.7	0.7 ± 0.4	0.5 ± 0.6
Copper	0.8 ± 0.9	3.9 ± 1.9	1.5 ± 0.9	1.3 ± 1.0	1.3 ± 1.1	1.1 ± 0.9	1.3 ± 0.9	1.1 ± 1.1
Zinc	1.7 ± 1.1	15.2 ± 7.9	3.1 ± 3.4	11.6 ± 14.0	3.4 ± 5.1	7.6 ± 11.9	3.9 ± 3.9	7.0 ± 12.5
Arsenic	8.2 ± 5.9	28.6 ± 26.9	8.3 ± 7.2	11.1 ± 7.2	14.1 ± 6.1	4.3 ± 3.1	7.1 ± 5.7	11.3 ± 7.3
Cadmium	0.00 ± 0.01	0.05 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0.01 ± 0.01	0.02 ± 0.01
Antimony	0.1 ± 0.1	1.6 ± 1.0	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.1
Dry biofilm (µg/g)								
Chromium	607 ± 608	238 ± 110	323 ± 169	160 ± 93.5	418 ± 435	308 ± 379	541 ± 516	186 ± 96.5
Nickel	468 ± 301	619 ± 88.7	540 ± 153	618 ± 584	679 ± 483	405 ± 200	551 ± 199	533 ± 521
Copper	252 ± 99.6	463 ± 118	288 ± 125	485 ± 607	476 ± 492	207 ± 74.5	259 ± 93.2	425 ± 511
Zinc	867 ± 343	1628 ± 221	1435 ± 410	5738 ± 10767	4162 ± 9074	1198 ± 456	1321 ± 334	4039 ± 9118
Arsenic	826 ± 549	1426 ± 720	2150 ± 2299	1118 ± 749	2052 ± 1877	677 ± 526	1029 ± 562	1700 ± 2050
Cadmium	5.5 ± 3.3	14.2 ± 2.4	10.6 ± 5.5	24.8 ± 37.7	18.8 ± 32.1	8.5 ± 5.1	9.6 ± 5.1	17.7 ± 32.4
Antimony	0.8 ± 0.4	3.5 ± 2.6	0.9 ± 0.7	3.4 ± 6.4	2.6 ± 5.4	0.9 ± 0.4	0.9 ± 0.5	2.5 ± 5.4
Number of samples	2	1	2	2	6	6	6	6

Table 4. Results of the Linear Mixed-Effects Model (LME) for metal and metalloid concentrations in water and dry biofilms: effects of season, relative position to the dam and precipitation regime (P.R.). The degrees of freedom are 1 for position, precipitation regime and their interaction, and 2 for season and its interactions with position and precipitation regime. Variables were transformed using $\log_{10}$, square root or Box-Cox to reach normality. Significant results ($P<0.05$) are in boldface.

	Season		Position		P.R.		Season*Position		Season*P.R.		Position*P.R.		Season*Position* P.R.	
Water	χ2	*P*	χ2	*P*	χ2	*P*	χ2	*P*	χ2	*P*	χ2	*P*	χ2	*P*
Chromium	65.54	**<0.05**	0.77	0.38	1.76	0.19	4.16	0.13	51.66	**<0.05**	5.21	**<0.05**	8.93	**<0.05**
Nickel	1.00	0.60	1.25	0.26	2.21	0.14	4.09	0.13	7.77	**<0.05**	0.01	0.94	3.53	0.17
Copper	4.13	0.13	0.34	0.56	1.02	0.31	5.02	0.08	5.79	0.06	2.73	0.10	2.08	0.35
Zinc	8.00	**<0.05**	2.51	0.11	0.07	0.79	4.98	0.08	13.46	**<0.05**	0.34	0.56	0.92	0.63
Arsenic	10.07	**<0.05**	83.49	**<0.05**	14.66	**<0.05**	1.09	0.58	40.89	**<0.05**	0.63	0.43	5.14	0.08
Cadmium	14.63	**<0.05**	0.65	0.42	0.13	0.72	3.23	0.20	13.26	**<0.05**	0.00	0.98	0.71	0.70
Antimony	16.10	**<0.05**	0.02	0.88	0.14	0.71	0.94	0.63	5.72	0.06	3.86	**<0.05**	0.14	0.93
Dry biofilm														
Chromium	10.63	**<0.05**	1.29	0.26	10.42	**<0.05**	1.36	0.51	11.22	**<0.05**	1.19	0.28	4.17	0.12
Nickel	1.37	0.50	6.46	**<0.05**	0.99	0.32	3.61	0.16	18.26	**<0.05**	1.95	0.16	9.29	**<0.05**
Copper	0.81	0.67	6.78	**<0.05**	0.42	0.52	1.93	0.38	2.84	0.24	1.64	0.20	1.66	0.44
Zinc	19.24	**<0.05**	3.68	0.06	0.05	0.82	0.16	0.92	10.51	**<0.05**	0.14	0.71	0.76	0.68
Arsenic	4.73	0.09	16.31	**<0.05**	0.01	0.96	0.51	0.78	7.60	**<0.05**	1.38	0.24	4.27	0.12
Cadmium	11.79	**<0.05**	0.60	0.44	0.42	0.52	1.99	0.37	12.17	**<0.05**	0.23	0.63	1.22	0.54
Antimony	3.67	0.16	0.39	0.53	0.35	0.55	1.90	0.39	8.93	**<0.05**	1.26	0.26	0.88	0.64

The precipitation regime had a significant interaction with the seasonal response of all the analysed abiotic variables, as well as for the concentrations of most of the analysed metals and metalloids (Table 1 to Table 4, Figure 5). All of the ecosystem structure parameters of biofilms and macroinvertebrates, with the exception of algal Shannon index and the organic matter percentage in biofilms, and the IASPT macroinvertebrate index, were affected by this interaction (Table 1 and Table 2). There was a remarkable pattern observed within this interaction during dry precipitation regimes in cold seasons, especially in winter. In this situation, there was marked water flow reduction coupled with an increase of algal and macroinvertebrate biomass, but also with a loss of algal and macroinvertebrate biodiversity (Figure 5).

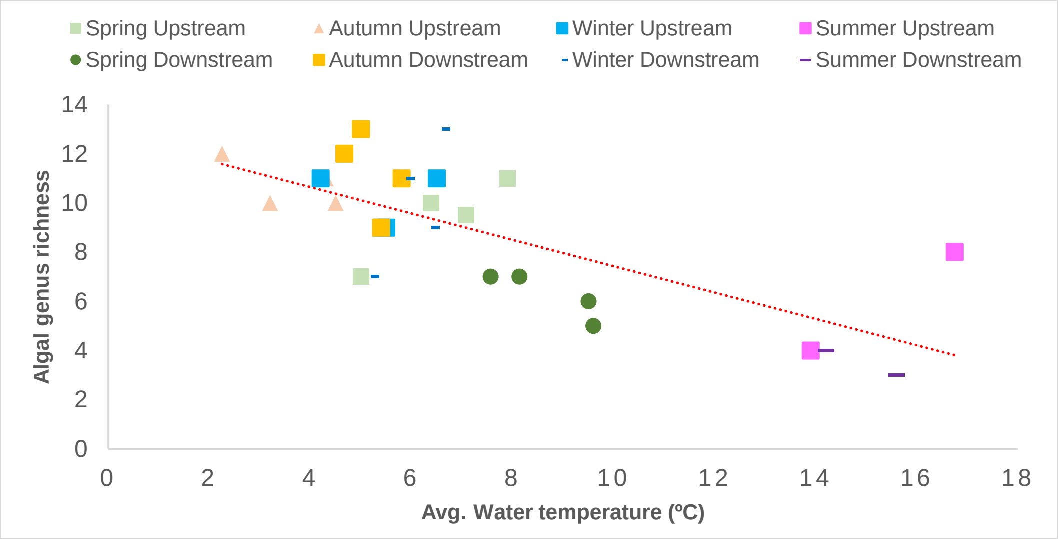

Figure 3. Algal genera richness in biofilms versus average water temperature of the stream between seasons and relative position from the dam (Upstream and Downstream).

3.4. Effects of the interaction between damming and the precipitation regime

The interaction between the precipitation regime and the relative position from the dam affected the water temperature, conductivity, oxygen concentration and FHI, as well as the water concentration in water of Cr and Sb. It also affected the autotrophic index of the biofilm and the macroinvertebrate biomass (Table 2 and Table 4). During the dry precipitation regime, it was observed downstream of the dam a higher water temperature and macroinvertebrate biomass, with a lower autotrophic index of the biofilms (Figure 6).

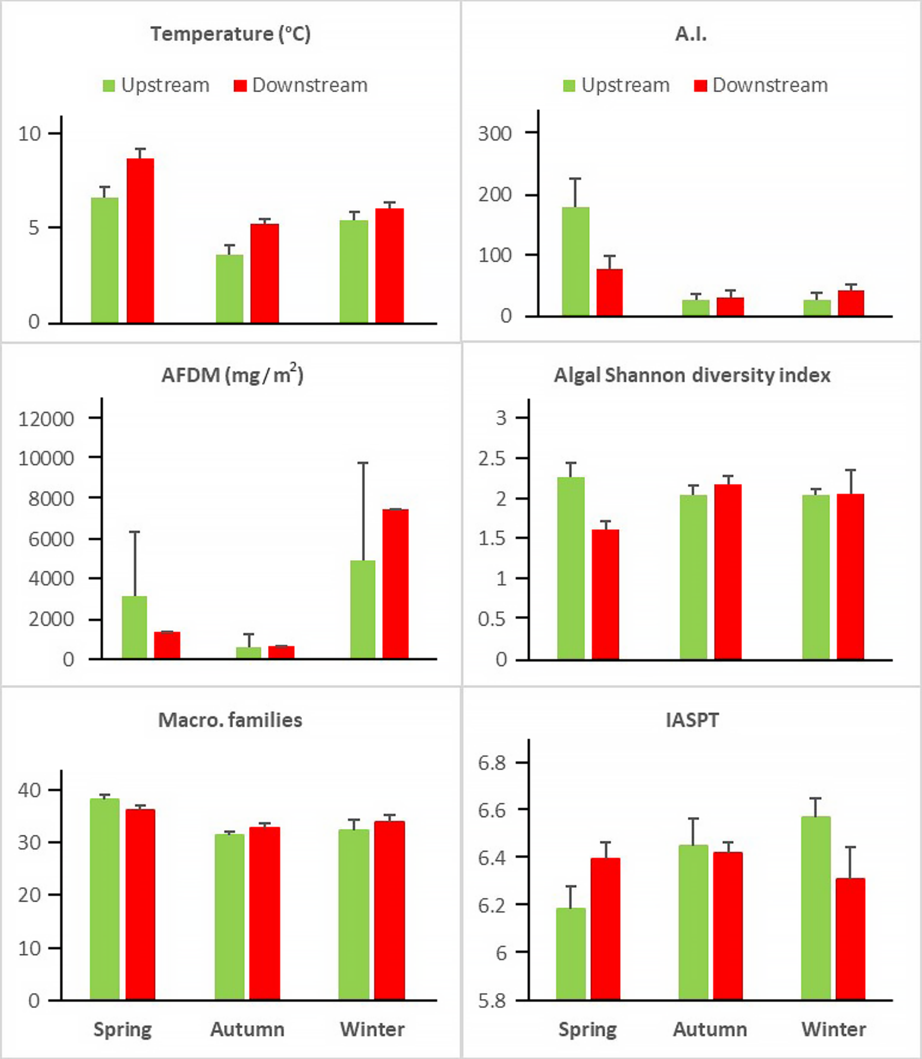

Figure 4. Effects of position from the dam for each season on the average values (with standard error) of water temperature, biofilm's autotrophic index (A.I.), biofilm's AFDM, algal Shannon diversity in biofilm; macroinvertebrate number of families and the Iberian Average Score per Taxon (IASPT) quality index. The effects of position on these variables differ significantly between seasons (see Table 2).

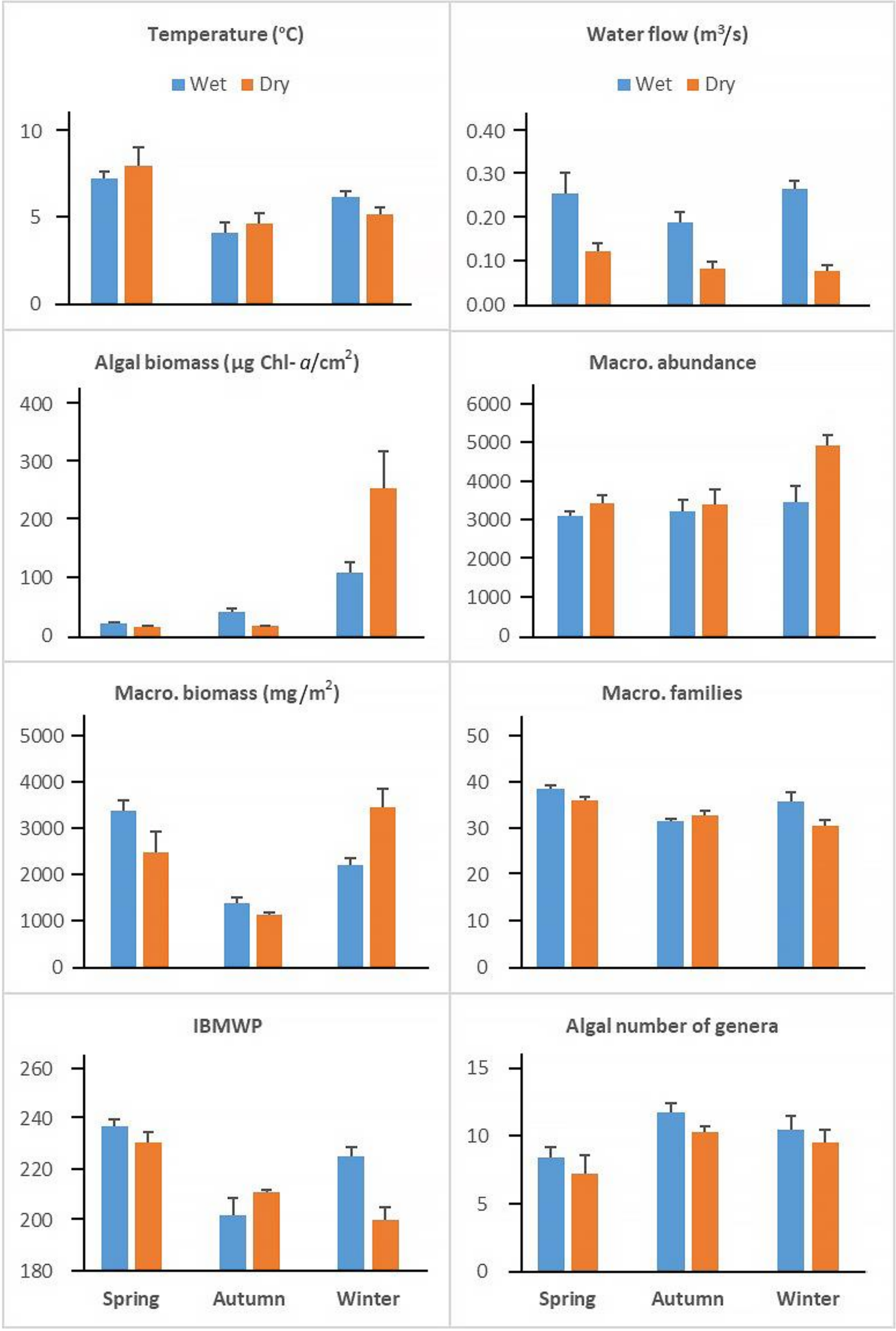

Figure 5. Effects of precipitation regime for each season on the average values (with standard error) of water temperature, water flow, algal biomass, algal number of genera, macroinvertebrate biomass and abundance, macroinvertebrate number of families and the Iberian Biological Monitoring Working Party (IBMWP) quality index. Notice that the effects of the precipitation regime on these variables differ significantly between seasons (see Table 2).

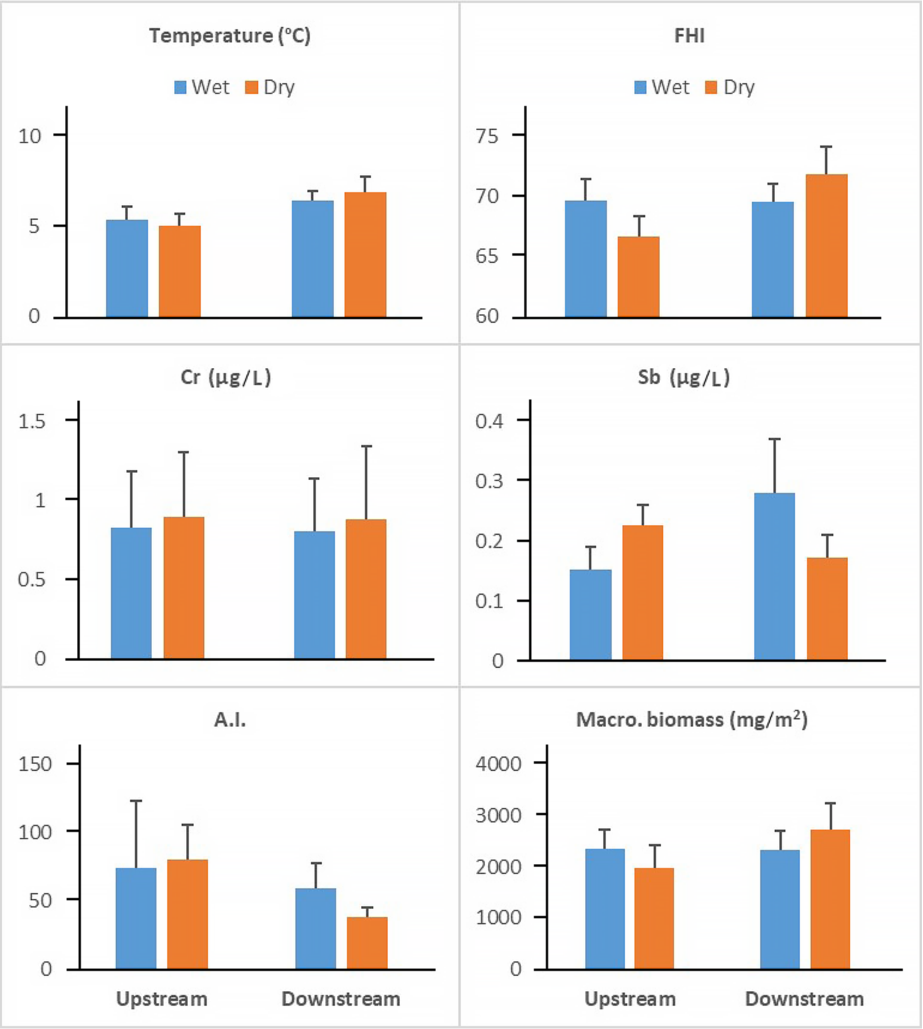

Figure 6. Effects of precipitation regime for each position on the average values (with standard error) of water temperature, fluvial habitat index (FHI), chromium (Cr) and antimony (Sb) concentrations in water, biofilm's autotrophic index (A.I.) and macroinvertebrate biomass. Notice that the effects of the precipitation regime on these variables differ significantly between positions (see Table 2 and Table 4).

4. Discussion

The structure and function of stream ecosystems is mainly driven by the hydrological regime (Maddock, 1999). In this case study, we observed that water flow was the main abiotic factor controlling the ecosystem structure of a headwater stream by having a great influence on the physical and chemical parameters of the water, which was also determined by other studies (Biggs et al., 2005; Sabater and Tockner, 2009; Dalu et al., 2017). The modulation of the water flow by factors like seasonality, damming and the precipitation regime had different effects on the ecosystem structure of the stream.

4.1. Ecosystem structure of headwater streams is driven by seasonality

The ecological structure of this headwater stream followed a marked seasonal pattern driven by the water flow and temperature. Winter was the most differentiated season because the cold temperatures and the low water flow due to the water retention in snow form allowed the bloom of the macroalgae *Hydrurus foetidus*, a rheophile and psychrophile chrysophyte algae that generates long filaments where many diatoms grow and macroinvertebrates thrive (Klavenes, 2019). The winter bloom of this macroalgae increased, on one hand, the algal production-related variables because of its macroscopic structure and, on the other hand, the algal genera richness because its thalli structure serves as a support for diatoms to grow. This primary production increase supposed a valuable food resource for emerging waterborne insect larvae (Milner, 2009), especially for chironomids (Klaveness, 2019), which promoted the winter peak of macroinvertebrate biomass and diversity. This winter production and biodiversity increase is key for the maintenance of the ecosystem structure of headwater streams with similar characteristics to the Catllar stream.

4.2. Antagonistic effects of damming and metal and metalloid pollution on the ecosystem structure of headwater streams

The presence of the dam implied a downstream water flow reduction that caused the physical and chemical parameters of the water to clearly differ from the upstream section of the stream. Despite this, the impacts of damming were weakly reflected on the ecosystem structure of the stream, which was also observed by Almeida et al., (2013) as macroinvertebrates are resilient to flow regulations. The water flow reduction and sediment retention caused by the dam increased the organic matter proportion of the biofilms (Sabater and Tockner, 2009), making them more digestible and attractive as a food resource for the macroinvertebrate community (Ceola et al., 2013), which promoted the macroinvertebrate biomass increase downstream of the dam. The water flow reduction caused by damming also had an indirect impact on the ecosystem structure of the stream through its interaction with the metal and metalloid pollution situation. Downstream of the dam, metal and metalloid concentrations were much lower despite the flow reduction because the particular disposition of the dam diverted the upstream water and caused most of the downstream water to come from an adjacent stream that was not impacted by metal pollution. This situation was reflected on the ecosystem structure with a downstream increase of the EPT index, as Ephemeroptera, Plecoptera and Trichoptera are macroinvertebrate groups sensitive to metal pollution (De Jonge et al., 2008) that can be replaced by others like Chironomidae in polluted streams (Li et al., 2010). The diversion of polluted water alongside the organic matter content increase in the biofilms promoted the downstream macroinvertebrate biomass and diversity increase. Consequently, damming and pollution had an antagonistic effect on the ecosystemic structure of the stream. Direct impacts of damming might not be strongly reflected on the ecosystem structure of headwater streams, but the water flow reduction that a dam implies could alter the physical and chemical characteristics of the downstream water, so indirect impacts on the ecosystem structure should be considered when assessing how damming affects headwater streams.

4.3. Synergic effects of seasonality with a precipitation decrease and damming on the ecosystem structure of headwater streams

During low water availability situations, the water flow of the stream decreased and its conductivity increased. This situation occurred during dry precipitation regimes and in winter due to the water retention in snow form. The interactive effects of a dry precipitation regime and the water retention in snow form during winter caused a synergic effect on the ecosystem structure of the stream by greatly reducing the water flow and increasing its temperature. This situation caused an increase on the biofilm and algal biomass, as well as on the macroinvertebrate biomass and abundance, but also resulted in an algal and macroinvertebrate diversity decrease. These results suggest that the algal biomass increase did not support a better-quality food resource for macroinvertebrates, causing a diversity and ecological quality loss with similar mechanisms as in eutrophication situations. In this case, the low flow conditions of the stream increase the water temperature, light penetration and nutrient concentrations in the water, promoting a shift to an autotrophic state and increasing the benthic biofilm biomass (Hilton et al., 2006; Yang et al., 2008; Sabater and Tockner, 2009; Dodds and Smith, 2016). The ecosystem structure of headwater streams requires a stable seasonal pattern of a cold and slow water flow in winter, and a faster flow in spring caused by the snowmelt. This biodiversity loss situation could be promoted by climate change through a warming of the stream water and a decrease in precipitations. Climatic models predict this situation on the Pyrenees (López-Moreno et al., 2008a), and similar cases could occur in headwater streams from other mountain ranges with similar characteristics. In this study, we observed that the water warming and biodiversity loss situation was also promoted due to seasonality and the precipitation regime synergically interacting with damming in low water availability situations. Consequently, the impacts of climate change on the ecosystem structure of headwater streams could be exacerbated in those affected by dams and other structures that reduce the water flow and increase its temperature.

4.4. Metal and metalloid pollution in headwater streams could be exacerbated in a climate change context

Most of the metals and metalloids found in the Catllar stream are commonly found in aquatic systems affected by wastewater from mining (Zhou et al., 2008; Guasch et al., 2009), and are known to cause changes in the structure of biofilms (Lawrence et al., 2004). It is known that algal production in biofilms declines with high As (Tuulaikhuu et al., 2015; Barral-Fraga et al., 2018) and Ni concentrations (Lawrence et al. 2004), among other metals (Zhou et al., 2008), and the As concentration in the water column and in biofilms in this stream was higher during the low precipitation regime due to the low water availability situation that it implies. In this situation, the stream presented a lower water flow that was warmer, more acidic and with a higher conductivity, and these factors promote the availability of metals and metalloids in the water column (Kar et al., 2008; Li et al., 2013). Climate change is expected to cause a decrease in precipitations and a water warming in the Pyrenees (López-Moreno et al., 2008b) as well as in other mountain ranges

(Jones and Rinehart, 2010; Bogan et al., 2014; Nukazawa et al., 2018), which could aggravate the impacts of metal and metalloid pollution on the ecosystem structure of headwater streams affected by mining by increasing their concentrations in biofilms and being transferred to higher trophic levels (Gümgüm et al., 1994; Barral-Fraga et al., 2020).

4.5. Recommendations for the conservation of headwater streams

Conservation of headwater streams is a fundamental consideration that needs to be addressed in order to effectively preserve freshwater ecosystems (Biggs et al., 2017). Since up to 90% of a river's flow is derived from headwater streams (Saunders et al., 2009), they play a vital role on whole river basins and are fundamental for the correct maintenance of the ecological integrity of a river network. In a climate change context, these ecosystems play a key role in river basins by providing water, ameliorating floods, transferring nutrients and carbon, intercepting pollutants, and supplying sediments (Biggs et al., 2017). Considering that the presence of dams is common on headwater streams from many mountain ranges, and that climate change will decrease the water availability in these ecosystems, the maintenance and restoration of a sufficient ecological flow in dam-impounded streams should be promoted (Rudra, 2018). More research is needed on the resistance and resilience of small headwater stream ecosystems to anthropogenic disturbances (Biggs et al., 2017), and our results highlight the importance of field studies in the assessment of how multiple anthropogenic stressors interact in an applied context.

5. Conclusions

This study case has showed the role of seasonality and the effects of both damming and a reduction in precipitations on the ecosystem structure of a headwater stream. These stressors had, by themselves, negative impacts on the biofilm and macroinvertebrate communities of the stream. They caused a water warming and flow reduction that promoted the autotrophy of the stream, increasing the algal and macroinvertebrate biomass but causing a biodiversity and ecological quality loss in a process similar to eutrophication. Additionally, both stressors had a synergic interaction with the water retention in snow form that occurs during the cold seasons by exacerbating those impacts, threatening the winter high levels of production and biodiversity that sustain a correct ecosystem structure on headwater streams.

Many streams are affected by metal and metalloid pollution, and our study case allowed us to understand that their ecological integrity is especially threatened by the water flow reduction that cold seasons and low precipitation regimes imply, as it can increase the concentration of metals and metalloids on the water column and in the biofilms. Despite this, damming can also diminish the impact of pollution situations by diverting the polluted water, thus increasing the downstream water quality. In summary, the maintenance of a stable ecosystem structure in headwater streams can be threatened by damming in the current climate change context with an expected water flow decrease and temperature increase in many mountain ranges, especially in headwater streams simultaneously affected by metal and metalloid pollution.

Chapter 2

Macroinvertebrate community responses to flow reduction in a dammed Pyrenean headwater stream

López-de Sancha, A., Roig, R., Jiménez, I., Vila-Gispert, A. and Guasch, H. (2022). Macroinvertebrate community responses to flow reduction in a dammed Pyrenean headwater stream. Submitted to International Review of Hydrobiology.

1. Introduction

Headwater streams are unique environments characterized by particular hydrological conditions: cold and oligosaline waters with conspicuous seasonal variations in the water flow (Milner and Petts 1994; Giller and Malmqvist 1998), which is the major driver of their ecosystem structure and function by controlling the physical and chemical attributes of waters (Bednarek 2001; Mor et al. 2018). The channelization and damming of headwater streams can reduce their water flow, impairing their structure and function and threatening their conservation (Mor et al. 2018; Zarfl et al. 2019). In order to properly manage and conserve these ecosystems and assure the goods, services and energy that they provide to human populations, it is necessary to understand how the alteration of the normal seasonal flow pattern can compromise the ecosystem structure and function of headwater streams (Maddock 1999; Bednarek 2001; Freeman et al. 2007; Dudgeon 2010).

Water flow reduction in dammed headwater streams could be exacerbated by the decreased precipitation expected by climate change in many regions. A clear example of this situation can be found on the headwater streams of the Pyrenees, in the Mediterranean basin, which are especially vulnerable to climate change. Climatic models expect here, by the end of the 21st century, a decrease in precipitations of between 10.7 and 14.8% and a mean increase in temperatures of between 2.8 and 4.0°C (López-Moreno et al. 2008), as well as a decrease in the maximum accumulated snow (López-Moreno et al. 2009). The ecosystem structure of Pyrenean headwater streams relies on a strong seasonal flow variability regulated by winter snow accumulation and spring snowmelt (Biggs et al. 2005; Sanmiguel-Vallelado et al. 2017). Climate change might impair this seasonal pattern by reducing winter snow cover and total precipitations, and by increasing stream water temperature (Cramer et al. 2018), causing slower and warmer water flows (OPCC-CTP 2018). Since freshwater organisms are adapted to a seasonal flow regime (Poff et al. 1997), these alterations can lead to strong impacts in the ecosystem structure and function of headwater streams (Perkins et al. 2010).

Benthic macroinvertebrates are a good tool to assess the effects of flow reduction (Bona et al. 2008; Resh 2008; Li et al. 2010), as the structure of their community assemblage changes in response to environmental disturbances in predictable ways (Boyle and Fraleigh 2003). If the morpho-behavioural mechanisms of food acquisition of the community assemblage are analysed, macroinvertebrates can be categorised by their functional status as Functional Feeding Groups (FFGs), providing information about both the structure and function of an ecosystem. Because the availability of food resources changes through space and time, the ratios between multiple FFGs can be altered seasonally and under different anthropogenic stressors (Cummins 1974; Merrit et al. 2002; Merrit and Cummins 2006; Masese et al. 2014; Wernersson et al. 2015).

Considering the possible impacts of water flow reduction, promoted by damming and climate change, on the ecosystem structure and function of Pyrenean headwater streams, and the potential of benthic macroinvertebrates to assess them, this study aims to use the macroinvertebrate community assemblage of a Pyrenean headwater stream to understand, on one hand, the seasonal pattern of its ecosystem structure and function and, on the other hand, how the water flow reduction caused by damming and by a precipitation decrease can impair that pattern. We hypothesize that water flow reduction will promote the autotrophy of the stream, having an impact on the ecosystem structure of the stream by reducing the biodiversity of the macroinvertebrate community and altering the proportion of functional feeding groups, promoting the herbivorous groups and reducing the abundance of predators.

2. Methodology

2.1. Study site and data collection

The study area is the same stream used in the first chapter of this thesis. It is a small Pyrenean headwater stream named Catllar, tributary of the Ter River (Catalonia, NE Iberian Peninsula), located between 1200 and 1600 m.a.s.l. We selected four sampling points (Upst 1, Upst 2, Down 1 and Down 2) with a similar separation between them, representative of the altitudinal zonation of the stream (General materials and methods: Figure 1). A hydroelectric dam was located between Upst 2 and Down 1 sampling points. It deviated most of the upstream water to a hydroelectric station, causing downstream water to come mainly from an adjacent stream (General materials and methods: Figure 2). We sampled macroinvertebrates at each sampling point seasonally for two years, between spring 2018 and winter 2020, resulting in eight samplings per point. For the interpretation of the results of the current study, we used the water and biofilm data from the first chapter of this thesis.

2.2. Macroinvertebrate samplings and analyses

Macroinvertebrate samplings were based on the quantitative protocols from the Catalan Water Agency (ACA, 2006) and the Spanish Ministry of Agriculture, Food and Environment (MAGRAMA, 2013). For each sampling point, a coverage percent estimation for each type of microhabitat substrate was assessed. Ten sampling units, each one covering 0.125 m^2, using a 25 cm wide and 500 µm mesh size hand net, were taken at each point proportionally distributed for each microhabitat type: one sampling unit per each 10% of microhabitat coverture, plus an extra half sampling unit for really minority habitats (<5%). Samples were stored in plastic containers with ethanol 80% until their processing and analysis at the laboratory.

Macroinvertebrates were identified in the lab using a stereoscopic microscope (Optika SZR-10) by means of identification keys (Campaioli, 1999; Malicky, 2004; Tachet et al., 2010; Oscoz et al., 2011). Taxonomic resolution was based on the IBMWP (Iberian Biological Monitoring Working Party) biological quality index (Alba-Tercedor et al., 2002) in which taxa were identified up to family level, except for Nematoda, Nematomorpha, Oligochaeta, Ostracoda, Hydracarina and Collembola, which were identified to those cited taxonomic levels. For each sample, a fraction of at least 300 individuals was identified and counted. All the individuals of the taxa that were not present in the analysed fraction, but were found in the rest of the sample, were also counted. Abundance of each taxon was calculated by extrapolating the abundance of individuals present in the analysed fraction plus the number of individuals in the rest of the sample, and used to assess their biomass. The dry mass of each taxon in the samples was assessed using a representative sub-sample of each taxon dried at 60°C, until constant weight, using a drying oven (Raypa DOD-20) and weighted (± 0.01 g) using an analytical scale (Sartorius Practum 124-1S). For biomass calculations, sub-samples that did not reach the detection limit (DL = 1 mg) a value of 0.7·DL was assigned. Samples with 30% of the taxa under the detection limit were not considered (Bennet, et al. 2000). Total weight of each sub-sample was divided by their total number of individuals. These values were multiplied by the abundance of each taxon and expressed as mg of dry mass/m^2. Finally, three Biological Quality Indexes for macroinvertebrates were calculated, which use the abundance of certain macroinvertebrate groups to evaluate the state of the ecosystem: IBMWP (Iberian Biological Monitoring Working Party) (Alba-Tercedor et al., 2002), IASPT (Iberian Average Score per Taxon) (Armitage et al., 1983), and EPT (Ephemeroptera, Plecoptera and Trichoptera Index) (Lenat, 1988).

2.3. Functional Feeding Groups

Macroinvertebrates found were assigned to FFGs using the trait classification proposed by Tachet et al. (2000), still widely used nowadays (Mello et al., 2020; Pastorino et al., 2020; Wang et al., 2020), where each taxon had a score assigned describing their affinity to each category of adaptations used to harvest nutritional resources. We selected the highest and most common affinity to a FFG for each macroinvertebrate taxa found, and classified it as predator (preys on living animal tissue), parasite (living in or on another organism), scrapper (feeds on periphyton), deposit feeder (collector: sediment detritivore), filter feeder (collector: suspension detritivore), shredder (feeds on living or decomposing plant tissue and on coarse particular organic matter (CPOM)) or piercer (sucks content of algal cells). It is important to note that within each FFG, there are obligate and facultative members, and these can be different species or different stages in the life cycle of a given species. Consequently, the most reliable linkage between a food resource category and macroinvertebrates is with the obligate forms in later instars (Merritt and Cummins, 2006).

Ratios between different FFGs were calculated in order to estimate different ecosystem function attributes of the stream following the criteria based on Merrit and Cummins (2006) (Table 1). This approach focuses on function rather than on taxonomy, so it is linked to the environmental gradient in water systems, reflecting its habitat quality and the availability of nutritional resources to which the macroinvertebrates respond. It represents the integration of the ecosystem structure and function in a single sampling, making this approach a fast and integrative technique (Merritt et al., 2002; Cummins et al., 2005; Masese et al., 2014; Mangadze et al., 2019).

Table 1. Ecosystem function parameters of the stream assessed from macroinvertebrate Functional Feeding Groups (FFGs). Adapted from Merrit and Cummins (2006).

Ecosystem function parameter	FFG ratios	General criteria ratio levels
Autotrophy to Heterotrophy Index	Scrapers to Shredders + Total collectors	Autotrophic if **>0.75**
Coarse Particulate Organic Matter (CPOM) to Fine Particulate Organic Matter (FPOM)	Shredders to Total collectors	Normal shredder association linked to functioning riparian system if Fall-Winter **>0.5** Spring-Summer **>0.25**
Suspended FPOM to Deposited in benthos FPOM	Filtering collectors to Gathering collectors	Suspended FPOM greater than normal particulate loading in suspension if **>0.5**
Substrate stability	Scrapers + Filtering collectors to Shredders + Gathering collectors	Stable substrate if **>0.5**
Top-Down predator control	Predators to Total all other groups	Normal predator to prey balance if value **between 0.10 and 0.20**

2.4. Statistical analyses

The precipitation regime of each season was highly different for each year in the Catllar stream, so in order to assess the potential effects of a precipitation reduction promoted by climate change, we used the average daily and accumulated precipitation data results from an in situ meteorological station and the water flow data from a nearby flow station in the Ter river (in the locality of Sant Joan de les Abadesses) (General Introduction: Figure 4) to categorize each season as Wet (spring 2018, summer 2019, autumn 2018 and winter 2020) or Dry (spring 2019, summer 2018, autumn 2019 and winter 2019), in relation to their opposite one.

To account for the influence of seasonality and assess the impact of damming and the different precipitation regimes on the macroinvertebrate community structure, each measured variable (global biomass, biomass for each FFG and biodiversity indices), was grouped by season (Spring, Summer, Autumn and Winter), relative position from the dam (Upst 1 and Upst 2= Upstream, Down 1 and Down 2= Downstream), and precipitation regime (Dry or Wet). These variables were checked and, if they did not meet normality and homoscedasticity assumptions, they were transformed using logarithms, square roots or Box-Cox transformations (Box and Cox, 1964). We then applied a Linear Mixed-Effects model (LME) for each variable by considering the season, relative position from the dam and precipitation regime as fixed effects, and the sampling point (Upst 1, Upst 2, Down 1 and Down 2) as a random effect.

In order to assess the differences in the macroinvertebrate community assemblage between seasons, relative position from the dam and precipitation regime, we performed an Analysis of Similarities (ANOSIM) using the *vegan* R package (Oksanen et al. 2019). The biomass of the macroinvertebrate taxa were used as dependent variables. Those taxa representing less than 1% of the total biomass where excluded due to their low contribution to the community. In addition, we also performed a Principal Component Analysis (PCA) of the biomass for each FFG on each sampling to analyse the differences between seasons and years. Finally, a non-parametric Multidimensional Scaling (nMDS) analysis was performed using the biomass of FFGs, and considering the stream water and algal variables analysed in the first chapter of this thesis (Chapter 1: Table 1) as the environmental factors, in order to assess the link between abiotic factors, producers and consumers on the structure and function of this stream. For this nMDS analysis, two seasonal replicates were considered, with the exception of summer. Summer 2018 had all the macroinvertebrate data available, but in regards to the abiotic and algal information, only the algal biovolume data was available. Summer 2019 presented complete information about all the parameters. All statistical analyses in this research paper were performed using the RStudio software version 1.2.5033 (RStudio Team, 2019).

3. Results

During the samplings, 58 different macroinvertebrate families were found (Table 2). Variables of biomass and diversity (number of families and the diversity indexes IBMWP, IASPT and EPT) were calculated using this data, and were then compared between seasons, relative position from the dam and precipitation regimes (Table 3 and Table 4). These macroinvertebrate families were also grouped as seven FFGs and their biomass was compared under those criteria (Table 5 and Table 6). Ratios between different FFG were used to calculate differences on the ecosystem function under the different analysed parameters (Table 6).

3.1. Seasonal pattern of the ecosystem structure and function of the stream

The biomass and biodiversity measures showed a seasonal pattern of the ecosystem structure of the stream with a macroinvertebrate biomass peak on winter and spring, being spring the season with the highest values of the IBMWP and EPT biodiversity indices (Table 3 and Table 4). The ANOSIM analysis showed that, when considered as FFGs, the macroinvertebrate community assemblage significantly differed between seasons (R=0.424, p=0.001). Shredders and scrappers were the most abundant fraction all year round, but the biomass of all FFGs differed between seasons (Table 5 and Table 6). The PCA analysis (Figure 1 and Figure 2) showed that shredders, scrappers, filter feeders and piercers were the FFGs that contributed the most to the variability observed in the macroinvertebrate community assemblage, and also showed that shredders and scrappers, as well as predators, were the dominant fraction during winter and spring.

The ecosystem function parameters analysed through the FFG ratios (Table 7) indicated that the stream was mainly autotrophic in winter and spring, and mainly heterotrophic in autumn and summer. This was related to the direction of the trophic chain, which was mainly top-down on winter and, marginally, in spring, and mainly bottom-up in autumn and summer. Finally, a normal shredder association, a suspended FPOM proportion higher than the sedimented one, and a stable riverbed was observed in all seasons.

3.2. Role of environmental conditions on the dominance of FFGs

The changes in the dominance of each FFG in each analysed situation were related to different environmental conditions. The nMDS analysis (Figure 3), performed with the data of the previous stream characterisation, showed the link of each FFG to the stream water and biofilm parameters considered. Physical and chemical variables of the water were the strongest parameters driving the ordinance of FFGs.

Table 2. Average biomass of macroinvertebrate families (mg/m^2) with their assigned trophic group found in the stream during seasons, relative position from the dam, and precipitation regime.

Macro. family	**FFG**	**Season**				**Position**		**Precipitation regime**	
		Spring	**Summer**	**Autumn**	**Winter**	**Upstream**	**Downstream**	**Wet**	**Dry**
Planariidae	Predator	13.9 ± 13.2	20.3 ± 30.1	3.5 ± 2.7	3.5 ± 3.9	13.7 ± 22.9	6.9 ± 8.6	9.0 ± 10.7	11.6 ± 22.5
Nematoda	Parasite	0.7 ± 1.0	0.3 ± 0.3	0.1 ± 0.2	0.1 ± 0.2	0.2 ± 0.2	0.4 ± 0.8	0.5 ± 0.8	0.1 ± 0.2
Nematomorpha	Parasite	0.0 ± 0.0	3.5 ± 6.1	0.0 ± 0.0	0.0 ± 0.0	1.6 ± 4.5	0.2 ± 0.9	1.8 ± 4.5	0.0 ± 0.0
Oligochaeta	Deposit feeder	67.0 ± 76.5	92.4 ± 128.0	15.4 ± 13.2	54.7 ± 77.4	73.6 ± 105.9	41.1 ± 55.8	73.9 ± 68.7	40.9 ± 97.9
Erpobdeliidae	Predator	22.4 ± 29.6	32.8 ± 38.4	3.6 ± 6.9	11.7 ± 18.7	22.1 ± 23.6	13.1 ± 30.6	28.9 ± 34.0	6.3 ± 10.5
Ancylidae	Scraper	5.3 ± 11.8	3.5 ± 7.0	0.1 ± 0.2	0.2 ± 0.4	0.7 ± 1.2	3.9 ± 9.5	4.5 ± 9.4	0.1 ± 0.2
Hydrobiidae	Scraper	0.3 ± 0.3	0.1 ± 0.2	0.2 ± 0.3	0.2 ± 0.2	0.2 ± 0.3	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2
Lymnaeidae	Scraper	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0
Sphaeriidae	Filter feeder	0.0 ± 0.0	0.1 ± 0.2	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.1	0.0 ± 0.1
Cladocera	Filter feeder	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.0
Ostracoda	Deposit feeder	0.1 ± 0.2	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.1
Niphargidae	Shredder	0.1 ± 0.2	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.1	0.1 ± 0.2	0.0 ± 0.0
Hydracarina	Predator	3.3 ± 2.0	6.8 ± 4.8	3.0 ± 1.7	3.2 ± 2.5	3.9 ± 3.0	4.3 ± 3.6	4.7 ± 4.0	3.4 ± 2.4
Collembola	Deposit feeder	0.1 ± 0.2	0.3 ± 0.3	0.0 ± 0.0	0.2 ± 0.4	0.1 ± 0.2	0.1 ± 0.3	0.1 ± 0.2	0.2 ± 0.3

(Continuation) Table 2. Average biomass of macroinvertebrate families (mg/m²) with their assigned trophic group found in the stream during seasons, relative position from the dam, and precipitation regime.

Macro. family	FFG	Season				Position		Precipitation regime	
		Spring	Summer	Autumn	Winter	Upstream	Downstream	Wet	Dry
Baetidae	Scraper	289 ± 115	96.1 ± 45.8	74.4 ± 50.4	280 ± 169	239 ± 164	130 ± 99.4	177 ± 137	192 ± 155
Ephemerellidae	Scraper	25.9 ± 18.3	47.9 ± 33.9	0.4 ± 0.8	2.7 ± 4.3	18.3 ± 30.5	20.2 ± 23.8	23.8 ± 31.4	14.7 ± 21.7
Heptageniidae	Scraper	565 ± 416	78.0 ± 34.3	242 ± 68.0	415 ± 285	286± 292	363 ± 323	374 ± 350	276 ± 255
Leptophlebiidae	Shredder	4.2 ± 2.2	5.2 ± 4.4	13.2 ± 16.7	20.7 ± 24.1	3.0 ± 3.2	18.6 ± 19.2	13.4 ± 19.8	8.2 ± 10.1
Chloroperlidae	Shredder	42.4 ± 21.1	1.6 ± 1.3	12.3 ± 13.9	41.6 ± 23.4	31.0 ± 28.0	18.0 ± 19.2	23.0 ± 24.5	25.9 ± 25.2
Leuctridae	Shredder	56.8 ± 10.6	9.7 ± 2.9	27.2 ± 16.1	73.9 ± 40.6	47.7 ± 38.0	36.0 ± 27.7	41.2 ± 29.3	42.6 ± 37.7
Nemouridae	Shredder	87.5 ± 34.2	60.9 ± 24.9	62.0 ± 39.9	183.5 ± 91.1	101.1 ± 71.6	95.9 ± 75.4	97.0 ± 31.5	100 ± 99.2
Perlidae	Shredder	60.1 ± 71.6	142± 185	85.9 ± 82.7	39.1 ± 58.7	88.0 ± 130.3	75.7 ± 96.8	51.1 ± 60.6	112 ± 143
Perlodidae	Predator	106 ± 69.8	19.3 ± 24.8	43.1 ± 31.5	131.± 97.5	106 ± 88.0	43.3 ± 44.3	79.7 ± 66.3	70.4 ± 86.2
Taeniopterygidae	Shredder	1.2 ± 3.1	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 2.9	0.8 ± 2.3	0.5 ± 2.0	1.1 ± 2.9	0.3 ± 0.8
Veliidae	Piercer	0.0 ± 0.0	0.5 ± 0.8	0.1 ± 0.2	0.0 ± 0.0	0.2 ± 0.6	0.1 ± 0.2	0.1 ± 0.2	0.2 ± 0.6
Curculionidae	Shredder	0.1 ± 0.4	0.1 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.4	0.1 ± 0.4	0.0 ± 0.0
Dytiscidae	Shredder	1.2 ± 2.0	4.0 ± 4.8	0.2 ± 0.6	1.4 ± 2.3	2.5 ± 3.9	1.0 ± 1.7	1.9 ± 3.3	1.5 ± 3.0
Elmidae	Scraper	180 ± 68.1	228 ± 144	83.3 ± 11.7	247± 308	144 ± 70.7	226 ± 237	164 ± 128	206 ± 217
Hydraenidae	Scraper	2.4 ± 0.9	12.5 ± 6.4	2.7 ± 1.5	3.8 ± 1.7	5.7 ± 6.1	5.0 ± 4.6	4.2 ± 3.6	6.5 ± 6.6

(Continuation) Table 2. Average biomass of macroinvertebrate families (mg/m^2) with their assigned trophic group found in the stream during seasons, relative position from the dam, and precipitation regime.

Macro. family	**FFG**	**Season**				**Position**		**Precipitation regime**	
		Spring	**Summer**	**Autumn**	**Winter**	**Upstream**	**Downstream**	**Wet**	**Dry**
Scirtidae	Scraper	0.7 ± 0.8	0.9 ± 1.2	6.6 ± 8.7	1.3 ± 1.3	3.4 ± 6.4	1.4 ± 2.7	4.0 ± 6.6	0.7 ± 0.9
Beraeidae	Shredder	0.1 ± 0.2	0.0 ± 0.0	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.0 ± 0.1	0.1 ± 0.2	0.1 ± 0.2
Brachycentridae	Shredder	17.7 ± 10.3	0.0 ± 0.0	7.3 ± 4.7	30.3 ± 27.1	14.4 ± 16.3	13.2 ± 20.3	8.1 ± 7.8	19.5 ± 23.4
Glossomatidae	Scraper	55.1 ± 93.2	5.2 ± 7.4	0.8 ± 0.7	13.4 ± 25.0	3.2 ± 4.9	34.1 ± 69.5	25.3 ± 68.9	12.0 ± 22.9
Goeridae	Scraper	8.7 ± 12.4	5.6 ± 6.8	1.6 ± 1.7	1.2 ± 1.4	2.2 ± 2.8	6.4 ± 9.9	4.9 ± 6.1	3.7 ± 8.8
Odontoceridae	Shredder	122 ± 99.7	69.5 ± 43.3	57.8 ± 35.0	92.2 ± 116	71.3 ± 90.4	99.5 ± 71.6	72.4 ± 70.0	98.4 ± 92.0
Philopotamidae	Filter feeder	24.0 ± 34.3	23.8 ± 20.0	14.1 ± 11.2	7.8 ± 15.7	7.8 ± 9.7	27.1 ± 26.8	17.0 ± 24.5	17.8 ± 20.2
Polycentropodidae	Predator	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0
Rhyacophilidae	Predator	81.5 ± 55.2	62.4 ± 108	31.3 ± 24.0	130 ± 82.2	93.0 ± 87.8	59.6 ± 68.8	78.2 ± 83.3	74.4 ± 78.1
Sericostomatidae	Shredder	7.7 ± 10.4	2.8 ± 5.1	0.4 ± 0.9	7.9 ± 16.4	6.2 ± 12.5	3.2 ± 6.9	8.2 ± 13.4	1.2 ± 2.2
Athericidae	Piercer	226 ± 239	174 ± 57.3	78.4 ± 52.6	158 ± 79.3	110 ± 79.8	208 ± 164	122 ± 68.6	196 ± 175
Blephariceridae	Scraper	1.1 ± 1.4	0.6 ± 0.4	0.1 ± 0.2	0.4 ± 0.7	0.9 ± 1.1	0.2 ± 0.2	0.7 ± 1.1	0.4 ± 0.5
Ceratopogonidae	Deposit feeder	1.7 ± 1.6	0.8 ± 0.6	0.0 ± 0.0	0.2 ± 0.3	0.7 ± 1.2	0.6 ± 0.8	0.7 ± 0.9	0.6 ± 1.2
Chironomidae	Scraper	59.7 ± 78.7	28.4 ± 38.4	2.0 ± 1.1	67.3 ± 58.1	39.4 ± 66.6	39.3 ± 46.6	32.2 ± 61.3	46.5 ± 52.3
Dixidae	Filter feeder	1.8 ± 3.0	2.6 ± 2.6	0.6 ± 0.4	0.5 ± 0.4	1.0 ± 2.0	1.7 ± 2.2	1.4 ± 2.2	1.4 ± 2.0

(Continuation) Table 2. Average biomass of macroinvertebrate families (mg/m^2) with their assigned trophic group found in the stream during seasons, relative position from the dam, and precipitation regime.

Macro. family	FFG	Season				Position		Precipitation regime	
		Spring	**Summer**	**Autumn**	**Winter**	**Upstream**	**Downstream**	**Wet**	**Dry**
Empididae	Predator	1.4 ± 1.3	1.2 ± 0.8	0.1 ± 0.2	0.4 ± 0.7	0.6 ± 0.7	1.0 ± 1.2	0.7 ± 0.7	0.9 ± 1.2
Ephydridae	Scraper	0.1 ± 0.2	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.2	0.1 ± 0.2	0.0 ± 0.0
Limoniidae	Shredder	20.7 ± 15.6	21.0 ± 18.0	13.2 ± 7.9	120 ± 85.6	29.7 ± 31.7	58.1 ± 80.6	35.8 ± 29.1	52.0 ± 83.3
Psychodidae	Shredder	4.7 ± 3.8	0.3 ± 0.3	0.8 ± 0.8	2.7 ± 2.0	3.0 ± 3.5	1.3 ± 1.2	1.6 ± 1.6	2.7 ± 3.5
Rhagionidae	Piercer	8.9 ± 7.9	10.5 ± 11.7	0.1 ± 0.2	0.6 ± 1.5	6.0 ± 10.4	4.0 ± 5.6	5.7 ± 9.1	4.4 ± 7.6
Simuliidae	Filter feeder	26.6 ± 26.7	6.8 ± 4.5	3.4 ± 2.0	30.4 ± 23.2	17.8 ± 22.8	15.8 ± 19.3	17.1 ± 22.4	16.5 ± 19.8
Stratiomyidae	Shredder	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1
Tabanidae	Piercer	0.0 ± 0.0	0.0 ± 0.0	10.8 ± 30.4	0.0 ± 0.0	5.4 ± 21.5	0.0 ± 0.0	5.4 ± 21.5	0.0 ± 0.0
Thaumaleidae	Scraper	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.1
Tipulidae	Shredder	10.2 ± 21.5	52.0 ± 26.6	128 ± 122	237 ± 250	91.1 ± 170	123 ± 151	154 ± 211	60.0 ± 57.0

Table 3. Average and standard deviation values of the macroinvertebrate biomass, number of families, and IBMWP, IASPT and EPT diversity indices grouped by season, relative position to the dam and precipitation regime.

	Season				Position		Precipitation regime	
Parameter	**Spring**	**Summer**	**Autumn**	**Winter**	**Upstream**	**Downstream**	**Wet**	**Dry**
Biomass (mg/m^2)	2931 ± 821	2052 ± 633	1258 ± 215	2840 ± 868	2155 ± 918	2384 ± 992	2207 ± 836	2333 ± 1070
Families	37.3 ± 1.8	37.6 ± 2.6	32.1 ± 1.7	33.1 ± 3.4	35.1 ± 4.1	34.9 ± 2.7	36.0 ± 3.4	34.1 ± 3.3
IBMWP	234 ± 7.2	224 ± 11.7	207 ± 9.8	213 ± 15.6	220 ± 17.8	218.7 ± 12.9	222 ± 15.6	216.5 ± 14.9
IASPT	6.3 ± 0.2	6.0 ± 0.2	6.4 ± 0.2	6.4 ± 0.3	6.3 ± 0.3	6.2 ± 0.3	6.2 ± 0.3	6.4 ± 0.3
EPT	17.9 ± 0.6	16.4 ± 0.9	16.6 ± 0.9	16.6 ± 1.4	16.7 ± 1.2	17.1 ± 1.1	17.0 ± 1.0	16.8 ± 1.2

Table 4. Results of the Linear Mixed-Effects Model (LME) for the macroinvertebrate biomass, the number of families and the IBMWP, IASPT and EPT diversity indices: effects of season, relative position to the dam and precipitation regime (P.R.). Degrees of freedom are 3 for season and its interaction with position and Precipitation regime, and 1 for the rest. Variables were transformed using log10, square root or Box-Cox to reach normality. Significant results ($P<0.05$) are in boldface.

	Season		Position		P.R.		Season*Position		Season* P.R.		Position* P.R.		Season*Position * P.R.	
Param.	χ2	*P*	χ2	*P*	χ2	*P*	χ2	*P*	χ2	*P*	χ2	*P*	χ2	*P*
Biomass	66.53	**<0.05**	1.74	0.18	0.22	0.63	3.05	0.38	18.62	**<0.05**	0.38	0.53	9.20	**<0.05**
Families	50.44	**<0.05**	0.03	0.85	7.98	**<0.05**	6.13	0.10	11.65	**<0.05**	0.01	0.92	1.15	0.764
IBMWP	41.12	**<0.05**	0.06	0.79	3.08	0.07	4.72	0.19	14.29	**<0.05**	0.17	0.67	1.70	0.635
IASPT	42.05	**<0.05**	0.12	0.72	9.06	**<0.05**	7.51	0.05	2.60	0.457	0.93	0.33	2.83	0.417
EPT	17.35	**<0.05**	0.72	0.39	0.78	0.37	10.05	**<0.05**	11.04	**<0.05**	0.19	0.65	2.16	0.538

The scrapper dominance was also highly related to the biofilm variables, and predators were mainly related to algal biovolume and biodiversity and the inorganic matter content of biofilms. Filter feeders and piercers were grouped similarly, associated directly with water conductivity, and inversely to water flow and the autotrophic index of the biofilms. Shredders were linked to the organic matter content in biofilms. Deposit feeders and parasites were the most differentiated FFGs but, as seen in the previous PCA analysis (Figure 1), they did not contribute much to the variability of the macroinvertebrate assemblage.

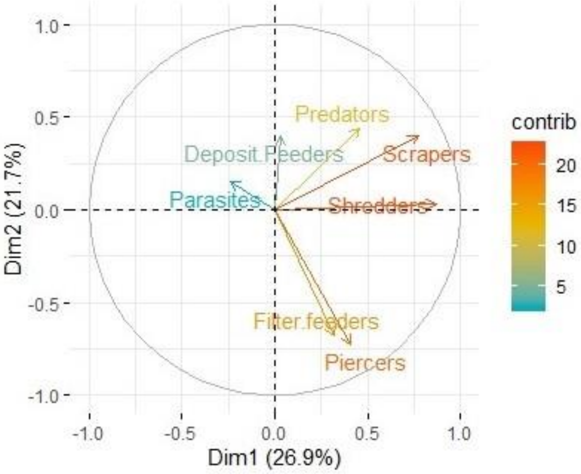

Figure 1. PCA indicating the relative contributions (contrib) of each macroinvertebrate Functional Feeding Group (FFG) to the variability of the macroinvertebrate community.

3.3. Precipitation regime effects on the macroinvertebrate community

In seasons with a dry precipitation regime, a slight decrease on the number of macroinvertebrate families was observed. In summer and winter under a low precipitation regime, the overall macroinvertebrate biomass increased and the biodiversity of its community decreased (Table 3 and Table 4). The ANOSIM analysis indicated no significant impact of the precipitation regime on the macroinvertebrate community assemblage using families, but its effects were reflected on the FFG analyses, with a lower biomass of parasites and deposit feeders during seasons on the dry precipitation regime (Table 6), indicating an effect on both the ecosystem structure and function. The interaction between seasonality and the precipitation regime showed multiple effects on the FFGs assemblage, which were especially reflected during spring (Figure 2). On one hand, the deposit feeders were almost absent in the dry winter and dry spring, but were highly abundant on the dry summer. On the other hand, scraper biomass increased on the dry winter, but decreased on the dry spring, when piercer biomass increased. This variability evidenced a more autotrophic stream, with a predominantly bottom-up trophic chain, during the dry precipitation regime (Table 7). Under both precipitation regimes, a normal shredder association, a suspended FPOM proportion higher than the sedimented one and a stable riverbed was observed.

3.4. Damming effects on the macroinvertebrate community

No direct effects of damming on the macroinvertebrate community were reflected using the general parameters of biomass and biodiversity, nor in the ANOSIM analysis, but the FFGs approach showed an impact of damming on the macroinvertebrate community assemblage and thus, on the ecosystem structure and function of the stream. Downstream of the dam, a lower predator biomass and a higher shredder and piercer biomass was observed, which implied changes on the functioning of the ecosystem as the analysed parameters indicated that downstream of the dam the direction of the food chain was mainly bottom-up, while being predominantly top-down on the upstream reach. On both positions, a normal shredder association, a suspended FPOM proportion higher than the sedimented one, and a stable riverbed was observed.

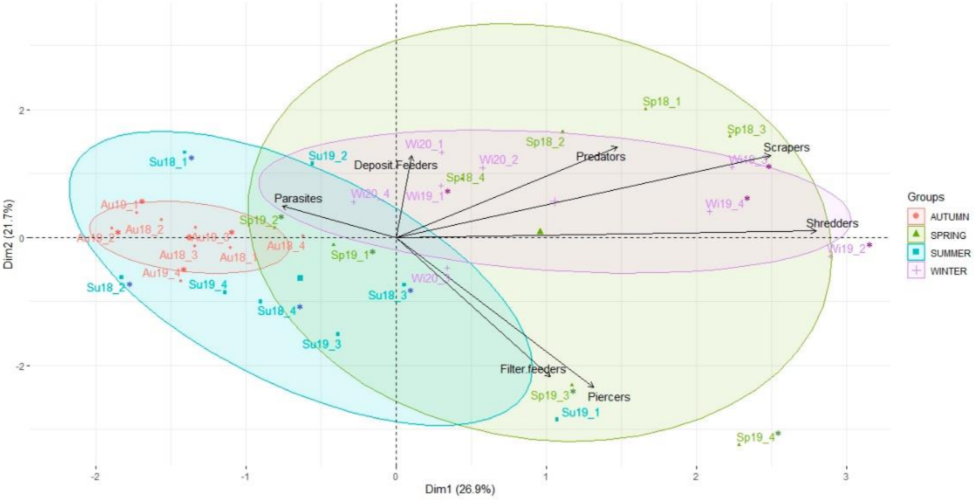

Figure 2. PCA plot showing the differences of the Functional Feeding Groups (FFGs) between seasons. Ellipses grouped the samples from each season (Sp= Spring, Su=Summer, Au = Autumn, Wi = Winter), year (2018, 2019 or 2020) and sampling points (1, 2, 3 or 4). Asteriscs indicate which sampling events were under the categorisation of “Dry”.

Table 5. Average and standard deviation values of the biomass (mg/m^2) of macroinvertebrate Functional Feeding Groups (FFGs) grouped by season, relative position to the dam and precipitation regime.

FFGs	**Season**				**Position**		**Precipitation Regime**	
	Spring	**Summer**	**Autumn**	**Winter**	**Upstream**	**Downstream**	**Wet**	**Dry**
Predators	229 ± 97.8	143 ± 142	84.6 ± 49.0	280 ± 123	240 ± 135	128 ± 96.5	201 ± 127	167 ± 132
Parasites	0.7 ± 1.0	3.9 ± 6.2	0.1 ± 0.2	0.1 ± 0.2	1.7 ± 4.7	0.7 ± 1.2	2.2 ± 4.6	0.1 ± 0.2
Deposit feeders	68.8 ± 76.9	93.5 ± 128	15.4 ± 13.2	55.1 ± 77.5	74.5 ± 106	41.9 ± 56.5	74.7 ± 69.2	41.7 ± 98.3
Scrapers	1194 ± 586	508 ± 192	415 ± 77.2	1035 ± 626	745 ± 448	831 ± 629	816 ± 564	760 ± 529
Filter feeders	375 ± 226	600 ± 367	177 ± 80.9	296 ± 149	370 ± 305	354 ± 241	286 ± 126	438 ± 351
Shredders	827 ± 360	519 ± 237	477 ± 224	1015 ± 281	603 ± 331	816 ± 344	694 ± 349	726 ± 361
Piercers	235 ± 236	186 ± 57.0	89.3 ± 44.2	159 ± 78.8	122 ± 75.8	213 ± 165	133 ± 62.9	201 ± 176

Table 6. Results of the Linear Mixed-Effects Model (LME) for the biomass of macroinvertebrate Functional Feeding Groups (FFGs): effects of season, relative position to the dam and precipitation regime (P.R.). Degrees of freedom are 3 for season and its interaction with position and precipitation regime, and 1 for the rest. Variables were transformed using log10, square root or Box-Cox to reach normality. Significant results (P<0.05) are in boldface.

FFGs	**Season**		**Position**		**P.R.**		**Season*Position**		**Season* P.R.**		**Position* P.R.**		**Season*Position * P.R.**	
	χ2	*P*	χ2	*P*	χ2	*P*	χ2	*P*	χ2	*P*	χ2	*P*	χ2	*P*
Predators	19.740	**<0.05**	5.370	**<0.05**	1.483	0.223	0.483	0.922	1.181	0.758	0.038	0.846	1.475	0.688
Parasites	33.493	**<0.05**	1.117	0.291	15.174	**<0.05**	0.754	0.860	33.692	**<0.05**	0.616	0.432	9.803	**<0.05**
Deposit feeders	9.032	**<0.05**	0.005	0.946	11.103	**<0.05**	3.929	0.269	18.319	**<0.05**	0.060	0.807	4.169	0.243
Scrapers	43.215	**<0.05**	0.183	0.669	0.318	0.573	1.881	0.597	18.693	**<0.05**	0.049	0.824	9.002	**<0.05**
Filter feeders	25.888	**<0.05**	0.050	0.824	1.922	0.166	1.600	0.659	5.440	0.142	0.313	0.576	2.539	0.468
Shredders	25.810	**<0.05**	6.713	**<0.05**	0.243	0.621	2.009	0.571	6.706	0.082	0.003	0.958	3.184	0.364
Piercer	11.813	**<0.05**	8.703	**<0.05**	1.286	0.257	5.412	0.144	8.202	**<0.05**	1.539	0.214	3.220	0.359

Table 7. Ecosystem function parameters calculated through the Functional Feeding Groups ratios using biomass data averaged by season, position and precipitation regime. Values that meet the previously considered criteria levels (Table 1) for each parameter are in boldface.

Ecosystem function parameter	Season				Position		Precipitation Regime	
	Spring	Summer	Autumn	Winter	Upstream	Downstream	Wet	Dry
Autotrophy to heterotrophy	**0.94**	0.42	0.62	**0.76**	0.71	0.69	**0.77**	0.63
Normal shredder functioning	**1.86**	**0.75**	**2.49**	**2.89**	**1.36**	**2.06**	**1.92**	**1.51**
Suspended to deposited sediments	**5.45**	**6.42**	**11.45**	**5.37**	**4.97**	**8.45**	**3.83**	**10.49**
Substrate stability	**1.75**	**1.81**	**1.20**	**1.24**	**1.65**	**1.38**	**1.44**	**1.56**
Top-down predator control	0.09	0.08	0.07	**0.11**	**0.13**	0.06	**0.10**	0.08

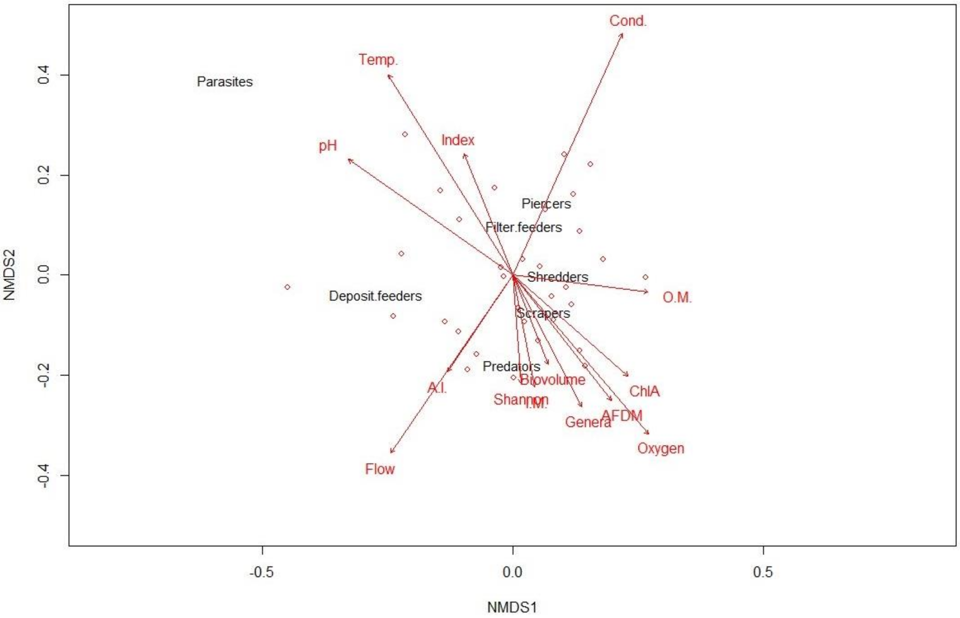

Figure 3. Non-metric multidimensional scaling (NMDS) showing the relationship between Functional Feeding Groups (FFGs) and environmental and algal variables previously measured. Abbreviations are: Cond. = Conductivity, Index= 430nm/665nm Absorbance Index, Temp. = Water temperature, pH= water pH, O.M.= Organic matter (%) in regards to inorganic matter in biofilms, ChlA= Chloropyll-*a* concentration in biofilm, AFDM = Ash free dry mass in biofilms, Oxygen = Oxygen saturation in water, A.I. = Autotrophic index, Genera = Number of algal genera in biofilms, Biovolume = Algal biovolume in biofilms, Shannon = Shannon's biodiversity index considering algae in biofilms, I.M. = Inorganic matter content in biofilms and Flow = Water flow.

4. Discussion

The macroinvertebrate community of the Catllar stream indicated that this headwater stream is a high-quality environment with high biodiversity scores all year round, dependent on a stable seasonal pattern, low temperatures and a natural hydrologic regime. The impacts of damming and a reduction in precipitations on the ecosystem structure and function of the stream were reflected on this community in multiple ways.

4.1. Seasonality role on the structure and function of Pyrenean headwater streams

Seasonality plays a key role on the ecology of headwater streams because their structure and function are mainly driven by the hydrological regime, which is strictly linked to seasonal patterns (Maddock 1999; Bona et al. 2008). Many of these streams are covered by deciduous trees, and the lack of leaves during winter and early spring allows more light to reach the streambed, when the water flow is shallower, slower and colder due to the water retention in snow form, promoting an increase in the biofilm and benthic macroalgae production (Romaní et al. 2012). This primary production bloom stimulates the high levels of macroinvertebrate biomass on spring and winter that were observed in our study (Romaniszyn et al. 2006; Milner 2009; Klaveness 2019; López-de Sancha et al., 2022). This seasonal pattern was also reflected on the ecosystem functioning, as during spring and winter, scrappers feeding on biofilms and macroalgae were the dominating fraction, indicating that the stream was mainly autotrophic during these seasons. This autotrophy promotes the dominance of herbivorous macroinvertebrate species, with short life cycles that allow their populations to turn over rapidly, renewing the food supply for the longer-lived predators that increase their abundance, as observed with the predator-to-prey functional ratio. In contrast, the stream was mainly heterotrophic on autumn and summer, when shredders and filter feeders dominated the macroinvertebrate fraction respectively. This fact reflects the dependence of biotic communities from headwater streams on the allochthonous organic matter inputs for a stable seasonal pattern, which comes mainly from the leaves of deciduous trees falling into the riverbed on those seasons. In this heterotrophic state, shredders convert CPOM particles from leaves into FPOM particles, which are used by filter feeders and other collectors (Merritt and Cummins 2006). The values of the CPOM:FPOM and the channel stability functional ratios indicated an abundant, high-quality source of this FPOM, coming from natural riparian and stream processes with a shredder action dominated by Tipulidae and Plecoptera taxa, which depend on fast leaf litter that is gone by spring (Vallania and Corigliano 2007). This autotrophic-heterotrophic seasonal pattern is thus needed in order to sustain the correct structure and function of Pyrenean headwater streams such as the Catllar, and its impairment through the water flow alteration promoted by damming and a precipitation reduction could threaten the conservation of these ecosystems.

4.2. Direct damming impacts on the macroinvertebrate community

Damming implies a downstream water flow reduction that has multiple effects on the macroinvertebrate community of headwater streams, as flow alterations act as filters for specific traits, with important consequences for stream processes (Bejarano et al. 2016). Water conductivity depends on the streamflow, and is one of the abiotic factors that contribute the most to the structure of stream macroinvertebrate communities (Karaouzas et al. 2019).

Water retention caused by the Catllar dam increased the ion and nutrient concentration of its waters and reduced the erosive forces on biofilms, favouring the autotrophy of the downstream reach. Both factors could promote the growth of algae and biofilms and increase their organic matter content, making them more digestible and a better food resource for the herbivorous macroinvertebrates, promoting their abundance (Franket et al. 2005; Sabater and Tockner 2009). The intensification of bottom-up trophic processes through this autotrophy promotion would alter the natural seasonal pattern of the macroinvertebrate community assemblage, reducing the strength of top-down processes and decreasing the predator abundance (Blanchet et al. 2008). Consequently, damming could have impacted the ecosystem structure and function of the downstream reach by favouring its autotrophy, altering the direction of the trophic chain (Merritt and Cummins 2006). However, other factors such as differences in the sunlight reaching the bottom of the stream, as well as different levels of leaf-litter inputs, could have also influenced this process. Moreover, the influence of the water coming from the adjacent tributary to the downstream reach could be limiting the interpretation of these results.

4.3. Impacts of a precipitation decrease on the ecosystem structure and function of Pyrenean headwater streams

A decrease in precipitations can reduce the water flow on headwater streams, impacting their ecosystem structure and function. In this situation, the presence of groups with certain traits favourable to these conditions would alter the natural seasonal assemblage of the macroinvertebrate community (Bogan and Lytle 2007; Bonada et al. 2007). Here, in dry periods, a biodiversity decrease and an alteration in the proportion of FFGs were observed. The lack of precipitation resulted in a reduction in water flow and in an increase of nutrient and ion concentrations, promoting the autotrophy of the stream and increasing the proportion of herbivorous groups and filter feeders, reducing the biodiversity of the stream (Ledger et al. 2011). This supposed an alteration of the direction of the trophic chain in a similar way as damming did, as the water flow was the main environmental factor impaired in both situations.

This altered condition of the natural seasonal pattern of the stream could be exacerbated on seasons and situations where the water availability is lower. Headwater streams that are threatened by a precipitation decrease would experience this situation frequently, impairing their ecosystem structure and function. Because of this, Pyrenean headwater streams are especially threatened by climate change, as a severe reduction in the precipitation regime and water availability is expected on this region during the current century (López-Moreno et al. 2008; López-Moreno et al. 2009). Headwater streams of similar characteristics that are also affected by damming would experience an even more severe water flow reduction, which could exacerbate these impacts on their ecosystem structure and function.

4.4. The use of macroinvertebrate FFGs as a link between ecosystem structure and function

The analysis of the macroinvertebrate community assemblage as FFGs has provided evidence of its utility as a link between the structure and function of headwater stream ecosystems (Cummins 1974; Merrit and Cummins 2006; Masese et al. 2014; Wernersson et al. 2015). We found that damming did not cause measurable changes in the general structural parameters of biomass and biodiversity, which is consistent with previous observations that macroinvertebrates are generally more influenced by the overall characteristics of a stream rather than by the local habitat (Hering et al. 2006). Despite this, damming inevitably alters the abiotic characteristics of a stream and, consequently, affects its food resources. This alteration did change the dominance of certain macroinvertebrate FFGs, showing the impacts of damming on both the structure and function of the ecosystem. Because of this, we encourage the use of both structure and function analyses on the assessment of anthropogenic impacts on headwater stream ecosystems, as they provide complementary, not redundant, information. The use of macroinvertebrate FFGs is a great tool to merge both approaches as it establishes a relationship between the stream environment, primary producers and consumers (de Figueroa et al. 2019), which is sensitive to the hydromorphological and biological pattern alongside the stream gradient and its alterations caused by both natural and anthropogenic stressors (Merrit and Cummins 2006).

5. Conclusions

Headwater streams follow a marked seasonal pattern that structures their macroinvertebrate community. Multiple families with different trophic strategies are substituted following the seasonal changes of water flow and light availability on the stream, which can affect them both directly and indirectly through the effects on the algal community. This pattern can be altered by the water flow reduction caused by both damming and a decrease in precipitations, which is currently being promoted by climate change. This situation can increase the nutrient and ion concentration in the stream, promoting its autotrophy and triggering a macroinvertebrate community shift that decreases the biodiversity of the stream, increasing the abundance of herbivorous groups while decreasing the abundance of predators, thus altering the direction of the trophic chain. The impacts of water flow reduction on headwater streams are best understood by using both ecosystem structure and function analyses as they provide complementary, not redundant, information, and an integrative assessment of both approaches can be achieved by using macroinvertebrate FFGs.

Chapter 3

Trophic competition in a guild of insectivorous semiaquatic vertebrates from a Pyrenean headwater stream: diet specialization of the endangered *Galemys pyrenaicus*

López-de Sancha, A., Roig, R., Aymerich, P., Vila-Gispert, A. and Guasch, H. (2022). Trophic competition in a guild of insectivorous semiaquatic vertebrates from a Pyrenean headwater stream: diet specialization of the endangered *Galemys pyrenaicus*. Submitted to Mammalian Biology.

1. Introduction

Headwater streams are unique habitats characterised by cold oligosaline waters, special hydrological conditions and fast water flow with conspicuous seasonal variability (Milner and Petts 1994; Giller and Malmqvist 1998). These fragile ecosystems are threatened by numerous anthropogenic stressors including habitat destruction, flow regulation and pollution (Bona et al. 2008; Dudgeon 2010), all of which can provoke a shift in the availability of certain basal ecosystem resources, thereby intensifying competitive interactions between the consumers of this type of resources (Biffi et al. 2017a). This is especially relevant in biodiversity conservation, which requires the effective application of good knowledge of the interactions between species and their environment. The ecological niche theory (Hutchinson 1957) states that sympatric species can coexist by exhibiting a degree of niche differentiation in prey or habitat use due to competitive interactions (Pianka 1974). Macroinvertebrates are one of the key basal food resources for many semiaquatic vertebrate predators in headwater streams (Allan 1995), which makes them potential competitors (Biffi et al. 2017a). For the effective conservation of these vertebrates, it is thus crucial to analyse their resource use and any potential niche overlaps with their competitors (Dudgeon et al. 2006).

The Pyrenean desman *Galemys pyrenaicus* (E. Geoffroy Saint Hilaire, 1811, Talpidae), endemic to headwater streams in the mountains of the Pyrenees and north of the Iberian Peninsula, is a semiaquatic vertebrate of great conservation concern. It is listed as Endangered by the IUCN (Quaglietta, 2021) as its populations are rapidly declining (Charbonnel et al. 2016; Aymerich and Gosàlbez 2018). It is known to be a generalist rheophile predator with a trophic preference for Trichoptera, Ephemeroptera and Plectoptera found in riffle sections of streams (Bertrand 1994; Santamarina 1993; Castién and Gosàlbez 1999; Esnaola et al. 2018). While some authors indicate that it also consumes a substantial amount of terrestrial prey (Gillet 2015; Biffi et al. 2017a; Esnaola et al. 2021), others suggest that in fact it consumes very little such prey (Santamarina 1993; Bertrand 1994; Castién and Gosálbez 1999; Hawlitschek et al. 2018).

Galemys pyrenaicus shares habitat and prey resources with other semiaquatic insectivorous vertebrates including the white-throated dipper *Cinclus cinclus* (Linnaeus, 1758) (Santamarina 1993; Bertrand 1994), a bird that consumes a great variety of prey items, with trichopterans and terrestrial prey known to be staples (Santamarina 1990; Santamarina 1993; Taylor and O'Halloran 2001). *G. pyrenaicus* also shares food resources with a number of species of water shrews depending on the location. In Pyrenean headwater streams, *G. pyrenaicus* co-exists with the Eurasian water shrew *Neomys fodiens* (Pennat, 1771) (Castién and Gosálbez 1999; Biffi et al. 2017a), which is also known to be a generalist predator in this aquatic environment that includes an important amount of terrestrial prey in its diet (Castién and Gosálbez 1999; Churchfield and Rychlik 2006). Hence, available information regarding the diet of these three semiaquatic vertebrates suggests that they all feed on stream macroinvertebrates, especially Trichoptera, Ephemeroptera and Plecoptera, although each has a preference for certain groups and includes terrestrial prey to some extent in its diet. It is also important to note that these species have different adaptations for capturing their prey. The Pyrenean desman has physiological and morphological features that afford it greater efficiency for capturing aquatic prey than the dipper or the water shrew. It is fully adapted to aquatic prey hunting and it can remain underwater for up to four minutes (Richard and Micheau 1975); by comparison, *N. fodiens* only submerges for up to 24 seconds (Mendes-Soares and Rychlik 2009) and *C. cinclus* for up to 30 seconds (Tyler and Ormerod 1988). Thus, the foraging efficiency provided by its diving ability allows *G. pyrenaicus* to specialise on aquatic prey due to the balance between the energetic costs of foraging and the energy provided by prey consumption.

Multiple studies have analysed the diet of the Pyrenean desman and are usually based either on the morphological or genetic identification of the macroinvertebrates found in its faeces; nevertheless, only a few studies have ever compared the interspecific trophic competition between *G. pyrenaicus* and other predators in the same habitat. Although some information on the diet overlap of this species with *N. fodiens* exists (Santamarina 1993; Biffi et al. 2017a), no studies have ever included *C. cinclus* in the comparison, despite its significant use of similar stream macroinvertebrates as a food resource (Santamarina 1990; Santamarina 1993). Considering that these three predators share a preference for similar macroinvertebrate groups, diet overlap and its consequent interspecific competition is to be expected. However, the physiological and morphological adaptations of *G. pyrenaicus* should make it more efficient at capturing aquatic prey and so diet specialisation on energetically profitable aquatic prey, as well as an avoidance of terrestrial prey, is likely to occur in this species. The aim of this study was thus to characterise the macroinvertebrate community of a Pyrenean headwater stream, describe the diets of the *G. pyrenaicus*, *N. fodiens and C. cinclus* individuals that live there, and assess their prey electivity and diet overlap. This information will enable us to improve our understanding of the trophic ecology of *G. pyrenaicus* and its diet specialisation, which, in turn, will help assess how anthropogenic stressors may impair access to key food resources and habitats for this species. We hypothesised that interspecific trophic competition will occur in these three semiaquatic predators, which will result in diet specialisation in *G. pyrenaicus* on aquatic energetically profitable prey.

2. Materials and methods

2.1. Study area

The study area was a Pyrenean headwater stream named Catllar, an influent of the Ter River, in Vilallonga del Ter, Catalonia (NE Iberian Peninsula), located between 1200 and 1600 m.a.s.l. We selected four macroinvertebrate sampling points (Upst. 1, Upst. 2, Down. 1 and Down. 2) (General materials and methods: Figure 1) considering a similar separation between them, representative of the altitudinal zonation of the stream. A small hydroelectric dam located between the Upst. 2 and Down. 1 sampling points divides the stream, deviating most of the upstream water to a hydroelectric station, so downstream water comes mainly from the adjacent west stream. Information about the water characteristics at each side of the dam was used from the previous results of this thesis (Chapter 1: Table 1).

2.2. Macroinvertebrate abundance samplings

Data about macroinvertebrate abundance was derived from the samplings performed in the Chapter 2 of this thesis. The macroinvertebrate abundance samplings were performed at each of the four sampling points once per season over a period of two years (spring 2018 to winter 2020; eight samplings per point), and followed the methodology of the quantitative macroinvertebrate sampling protocols used by the Catalan Water Agency (ACA 2006) and the Spanish Ministry of Agriculture, Food and Environment (MAGRAMA 2013). At each sampling point, the percentage coverage of each type of microhabitat substrate was estimated. Ten 0.125 m^2 sampling units were established at each point in proportion to the distribution of each microhabitat type: one sampling unit for each 10% of microhabitat coverage, plus an extra half sampling unit for minority habitats (<5%). Samples were taken using a using a 25-cm wide and 500-µm mesh-size hand net and then stored in plastic containers with ethanol 80% until processing and analysis in the laboratory.

Macroinvertebrates were identified using a stereoscopic microscope (Optika SZR-10) and identification keys (Campaioli 1999; Malicky 2004; Tachet et al. 2010; Oscozet al. 2011, Osorio et al., 2021). The taxonomic resolution was based on the IBMWP Biological Quality Index (Alba-Tercedor et al., 2002), in which taxa other than Nematoda, Nematomorpha, Oligochaeta, Ostracoda, Hydracarina, Collembola, Nemouroidea and Perloidea were identified to family level. For each sample, a fraction of at least 300 individuals was identified and counted. All the individuals of the taxa not present in the analysed fraction but present in the rest of the sample were also counted. The abundance of each taxon was calculated by extrapolating the abundance of all individuals present in the analysed fraction plus the number of individuals in the rest of the sample. The results were expressed as densities (individuals/m^2) based on the size of the sampled area.

2.3. Insectivorous semiaquatic vertebrates of the Catllar stream

The presence of the studied predators and their distribution in the Catllar stream had been determined previously (personal communication). Despite the coexistence of two water shrew species in the basin of the river Ter, only *N. fodiens* has ever been detected in the Catllar stream (via molecular analyses) and so all water shrew diet samples were attributed to this species. While *N. fodiens* was frequent along the whole length of the stream, the Pyrenean desman *G. pyrenaicus* was only found upstream of the dam. This subpopulation of Pyrenean desman forms part of the highly endangered desman population in the Alt Ter basin (NE extreme of its range), which is composed of small, somewhat isolated subpopulations. Although both mammals were detected all year round in similar abundances, the numbers of dippers *C. cinclus* fluctuated seasonally, and were commonest in spring and summer.

2.4. Diet samples

To determine the use of benthic stream macroinvertebrates *G. pyrenaicus*, *N. fodiens* and *C. cinclus*, diet samplings were performed three times per season between March 2018 and November 2021 (but not in 2020 or in winter), resulting in nine samplings per year. Six transects of 200 meters each, three below and three above the dam, were walked and diet pellets and depositions were visually searched for on projecting objects (i.e. rocks and roots) and in cavities. The macroinvertebrate sampling points were within the diet sampling transects. A standardised transect length of samplings for detecting *G. pyrenaicus* (Queiroz et al. 1998; Aymerich and Gosàlbez 2014) was used and is also appropriate for *N. fodiens* (Aymerich and Gosàlbez 2004), as repeated visits to the same sites increase the detection probabilities of both species (Charbonnel et al. 2014). The identification of the diet samples of each species was based on the expert criteria of the investigators taking into account where they were found and their size, colour and smell. The obtained samples were preserved in ethanol until analysis, when they were disaggregated using hydrogen peroxide, and macroinvertebrate mouthparts and key sclerites were separated, counted to determine the abundance of individuals per sample, and identified to the same taxonomic level as the macroinvertebrate abundance samplings. For identification, a reference collection of mouthparts and sclerites from the macroinvertebrate abundance samplings was used. Oligochaeta individuals were counted as a single individual when chaetae were found, while molluscs were identified through parts of their shields. All the data used for the prey electivity and trophic overlap analyses corresponded to the aquatic taxa found in the diet samples as no data on the availability of terrestrial prey was obtained.

2.5. Trophic overlap analyses

Aquatic prey counts were used to calculate the Pianka niche overlap index (Pianka 1974) to determine the degree of trophic overlap between the three analysed predator species in the stream (not including terrestrial taxa). This index ranges from 0 (no trophic resources used in common) to 1 (full dietary overlap). The same counts were used to generate a Venn diagram showing the number of different prey taxa unique to each predator, together with the taxa shared by these predators.

Ivlev’s electivity index (Ivlev 1961) was calculated using the Electivity R package (Quintans 2019). The average abundance of each prey item found in the diet samples for each predator was compared to its average availability in the stream. The resulting index value was represented as a heatmap with an average linkage clustering method and a Euclidean distance measurement method. This index ranged from -1, indicating that a prey item was abundant in the stream but avoided by the predators, to +1, indicating that the prey taxon was rare in the stream but preferred by the predators; 0 indicates random feeding. All analyses were performed using the RStudio software version 1.2.5033 (RStudio Team 2019).

3. Results

During the macroinvertebrate samplings, similar abundance densities were found in all seasons. The highest average abundance in the stream of taxa consumed by predators were the ephemeropterans Baetidae and Heptageniidae, and the water beetles Elmidae. Intermediate abundances of the plecopterans Nemuroidea, the trichopterans Hydropsychidae, Limnephillidae, Brachycentridae and Odontoceridae, and the ephemeropterans Ephemerellidae were also found (Figure 1).

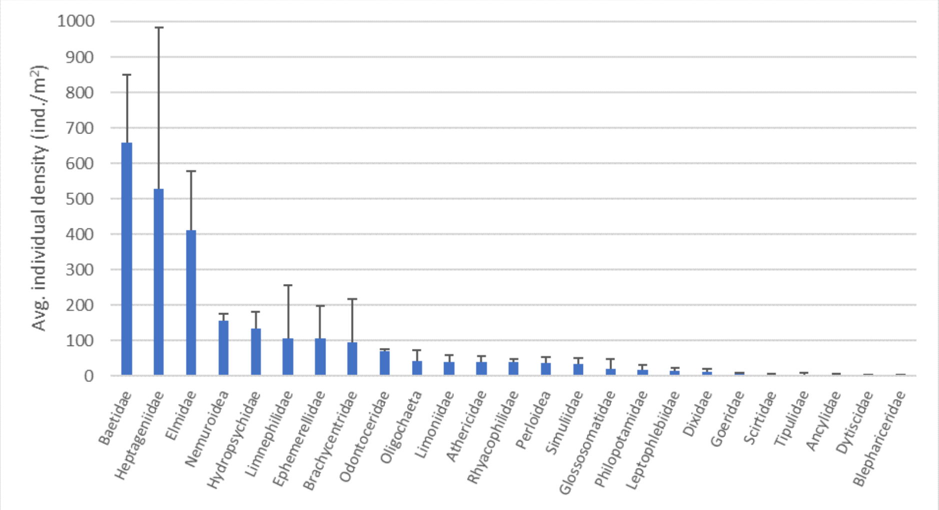

Figure 1. Average values (individuals/m^2), with standard error, of the macroinvertebrate prey availability in the Catllar stream found during the seasonal samplings (spring, summer and autumn). It includes only the availability of the terrestrial taxa consumed by the analysed vertebrate predators.

During the diet characterisation samplings, a total of 1996 prey items were identified in 75 samples, 1012 for *G. pyrenaicus* (28 depositions), 309 for *N. fodiens* (32 depositions) and 675 for *C. cinclus* (14 pellets). The average abundance values of prey taxa for each predator are shown in Table 1. The biomass of the total pellet was not estimated. The data indicate that the main components of the diet of *G. pyrenaicus* were ephemeropterans (33.1%) and trichopterans (29.5%). This species thus fed almost exclusively on taxa in aquatic life stages as less than 0.2% of its diet was composed of terrestrial prey. Similarly, the diet of *N. fodiens* was based on trichopterans (32.0%) and ephemeropterans (24.3%), but did also include a relevant proportion of terrestrial prey (10.0%). Both mammals included around 14–15% of dipterans and 17–18% of plecopterans in their diets. Finally, *C. cinclus* based its diet on trichopterans (51.1%) and ephemeropterans (30.7%), with just 1.9% of its diet consisting of terrestrial prey.

The Pianka niche overlap index based only on aquatic prey reveals that there was a clear diet overlap between *G. pyrenaicus* and *N. fodiens* (0.928). By comparison, the diet overlap of these two mammals with the dipper C. cinclus was not that strong (Pianka value = 0.446 in both cases; Table 2). A similar pattern was observed in the Venn diagram (Figure 2): all predators shared 14 prey taxa, *G. pyrenaicus* and *N. fodiens* shared five taxa, *N. fodiens* only shared one group with *C. cinclus*, and *G. pyrenaicus* shared two groups with the dipper.

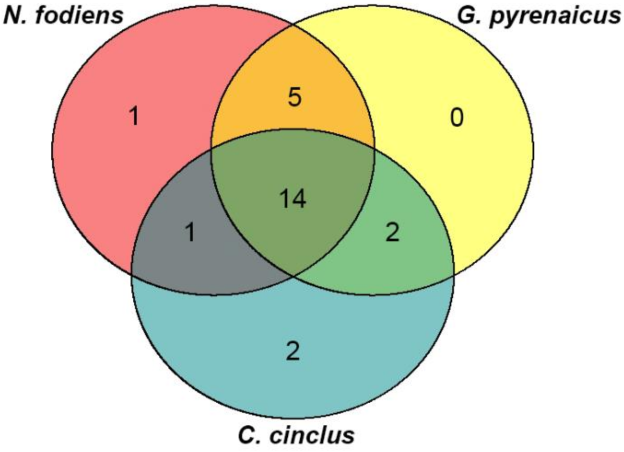

Figure 2. Venn's diagram that shows the number of unique and shared prey taxa found on the diet samples for each predator (*N. fodiens, G. pyrenaicus* and *C. cinclus*).

Table 1. Average abundance (individuals/sample), with standard deviation, of each taxon found in all the diet samples of *Galemys pyrenaicus*, *Neomys fodiens* and *Cinclus cinclus.*

Prey taxa		***G. pyrenaicus***	***N. fodiens***	***C. cinclus***
Oligochaeta	*Indeterminate*	0.07 ± 0.26	0.03 ± 0.17	-
Mollusca	Ancylidae	-	0.03 ± 0.17	0.93 ± 2.94
Ephemeroptera				
	Baetidae	7.28 ± 7.82	1.48 ± 2.29	2.50 ± 2.56
	Ephemerellidae	1.68 ± 2.45	0.21 ± 0.48	7.43 ± 8.46
	Heptageniidae	2.79 ± 2.83	0.51 ± 0.57	4.79 ± 6.59
	Leptophlebiidae	0.04 ± 0.19	0.06 ± 0.24	0.07 ± 0.26
	Indeterminate	0.18 ± 0.95	-	-
Plecoptera				
	Nemouroidea	4.64 ± 6.72	1.24 ± 2.42	1.86 ± 1.91
	Perloidea	1.93 ± 1.86	0.36 ± 0.55	3.14 ± 3.06
Coleoptera				
	Elmidae	1.11 ± 1.72	0.06 ± 0.24	1.00 ± 1.71
	Scirtidae	0.29 ± 0.66	0.06 ± 0.24	-
	Dytiscidae	-	-	0.21 ± 0.80
	Indeterminate	-	0.03 ± 0.03	-
Trichoptera				
	Brachycentridae	-	-	11.07 ± 36.41
	Glossosomatidae	-	0.03 ± 0.17	0.14 ± 0.36
	Goeridae	0.03 ± 0.19	0.03 ± 0.17	1.14 ± 1.99
	Hydropsychidae	4.32 ± 4.08	0.79 ± 0.99	2.00 ± 2.11
	Limnephilidae	0.71 ± 0.94	0.97 ± 1.99	3.71 ± 2.52
	Odontoceridae	3.96 ± 3.60	0.58 ± 1.22	2.14 ± 2.62
	Philopotamidae	0.61 ± 1.60	0.27 ± 0.94	-
	Rhyacophilidae	0.93 ± 1.05	0.21 ± 0.48	4.28 ± 2.84
	Indeterminate	0.07 ± 0.38	0.12 ± 0.54	0.14 ± 0.36
Diptera				
	Athericidae	0.04 ± 0.19	-	0.07 ± 0.27
	Blephariceridae	0.04 ± 0.19	-	-
	Dixidae	2.43 ± 3.99	0.63 ± 1.69	0.14 ± 0.53
	Limoniidae	0.25 ± 0.52	0.12 ± 0.33	-
	Simuliidae	1.32 ± 3.69	0.24 ± 0.61	0.21 ± 0.58
	Tipulidae	1.32 ± 2.88	0.24 ±0.61	0.29 ± 0.46
	Indeterminate	0.04 ± 0.19	0.09 ±0.38	-
Terrestrial groups				
	Miriapoda.	-	0.12 ± 0.33	-
	Heteroptera.	-	0.06 ± 0.35	-
	Coleoptera.	-	0.18 ± 0.46	0.21 ± 0.58
	Diptera	0.4 ± 0.19	0.06 ± 0.35	-
	Formicidae	-	0.18 ± 0.53	0.21 ± 0.58
	Araneidae	-	0.03 ± 0.17	-
	Indeterminate	-	0.03 ± 0.63	0.50 ± 0.76
Total consumed individuals		1012	309	675
Total consumed taxa		25	31	24
Number of diet samples		28	32	14

Ivlev's electivity index (Figure 3) compares the selection of aquatic prey by the three analysed predators and prey availability in the stream. All three actively shared and positively selected the trichopterans Odontoceridae and Rhyacophilidae, and the plectopterans Perloidea. However, many abundant taxa in the stream were not positively selected but were still actively consumed: the trichopterans Glossosomatidae, the ephemeropterans Heptageniidae and Baetidae, the dipterans Athericidae and Limoniidae, the coleopterans Elmidae, and Oligochaeta worms. Despite these similarities, there were notable differences between each predator in terms of their prey electivity.

Table 2. Results of the Pianka dietary niche overlap index between the mammals *G. pyrenaicus* and *N. fodiens*, and the bird *C. cinclus*. Values close to 0 indicate no trophic resources used in common, while values close to 1 indicate a full dietary overlap. These values do not include the proportion of terrestrial taxa.

Species compared	*Galemys - Neomys*	*Neomys - Cinclus*	*Cinclus - Galemys*
Pianka's Index value	0.928	0.446	0.446

C. cinclus clearly differed from the other predators as it actively selected groups such as the beetles Dytiscidae and the trichopterans Brachycentridae, Goeridae and Limnephillidae, and the ephemeropterans Ephemerellidae, as well as the molluscs Ancylidae that were avoided – or only randomly captured – by the mammals. It avoided scarce taxa such as Scirtidae and Philopotamidae. Conversely, although *G. pyrenaicus* and *N. fodiens* shared a positive electivity for many prey items, *N. fodiens* also positively selected the trichopterans Goeridae and Limnephillidae and the molluscs Ancylidae, which were not actively selected by *G. pyrenaicus* and only preyed upon somewhat randomly.

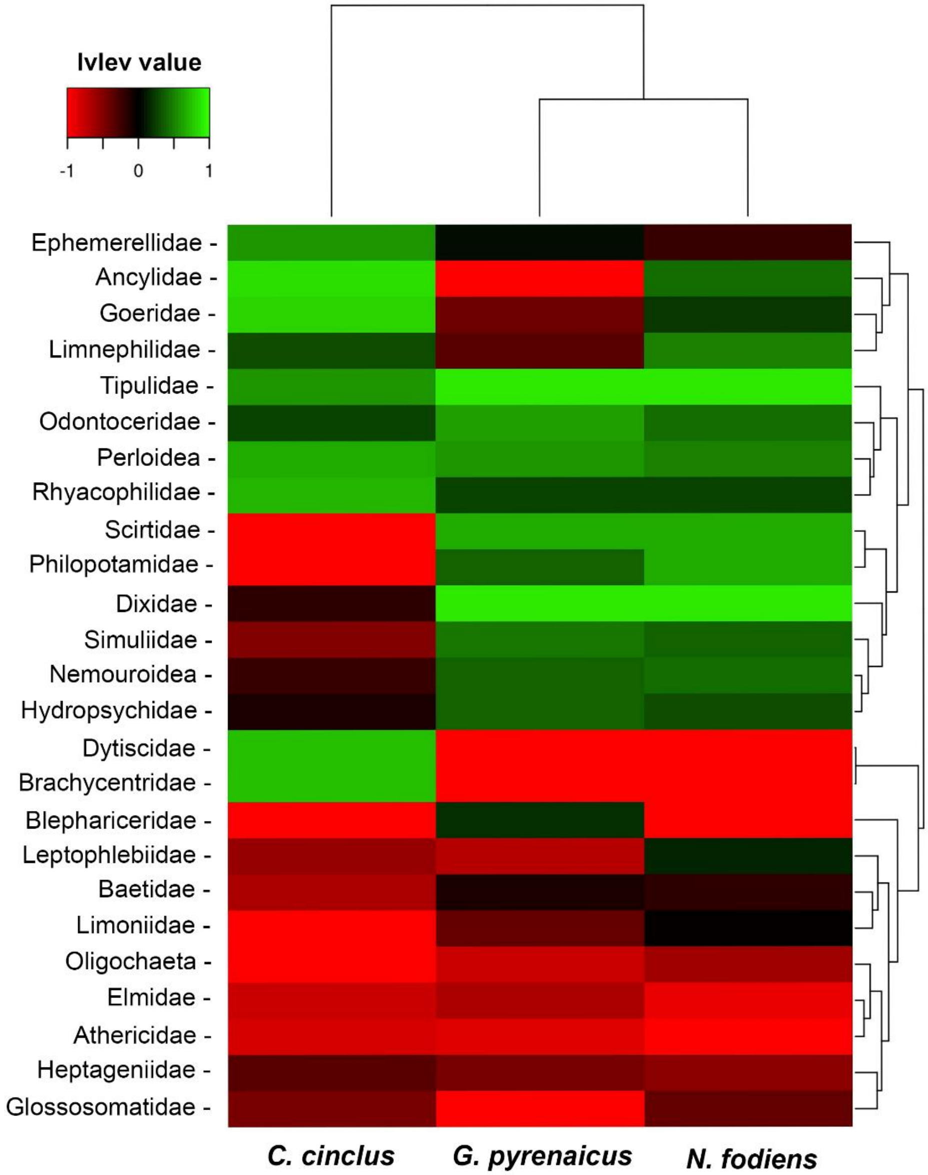

Figure 3. Ivlev's electivity index for the consumed macroinvertebrate prey by each predator (*N. fodiens, G. pyrenaicus* and *C. cinclus*), represented as a heatmap. The electivity ranges from -1 (red), indicating that a prey is abundant in the stream but avoided by the predator, to +1 (green), indicating that the prey taxon is rare in the stream but it is preferred by the predator. Values near 0 (black) indicate a random feeding.

4. Discussion

The diet analysis of these three stream vertebrates revealed a clear overlap that gives rise to interspecific trophic competition, especially between *G. pyrenaicus* and *N. fodiens*. General tendencies were observed including the negative electivity of groups that are scarce in the stream (e.g. Leptophlebiidae), had a low energy yield due to their low digestibility (e.g. Athericidae, Limoniidae), or belonged to highly chitinous (e.g. Elmidae) or hard-cased (e.g. Glossomatidae) families. Groups that were difficult to access due to their habitat selection (e.g. Blephariceridae, Oligochaeta) were also avoided (Tachet et al. 2010; Biffi et al. 2017a). By contrast, the prey groups that were positively or randomly selected by all the predators were rheophile, abundant and energetically profitable taxa, mainly trichopterans but also ephemeropterans, plecopterans and the large dipterans Tipulidae. Despite these similarities, certain differences in prey electivity by each predator were observed, which prompted resource partitioning based on morphological and physiological differences.

4.1. Prey electivity in the white-throated dipper (*C. cinclus*) and water shrew (*N. fodiens*)

The generalist diet of the dipper, which is known to contain trichopterans as a staple (Santamarina 1990; Santamarina 1993; Taylor and O’Halloran 2001), was reflected in the results of our study. This bird selected the most available and energetically profitable prey, and trichopterans were the most abundant fraction in its diet (i.e. high electivity values for families such as Goeridae, Limnephilidae, Rhyacophyllidae, Brachycentridae and Odontoceridae). Trichoptera larvae are the largest macroinvertebrate group (in terms of biomass) available to dippers and are used by dippers to feed their nestlings (Taylor and O’Halloran 2001), which would explain their active selection of this group. The dippers in this stream also used ephemeropterans as an important food resource: some of the families of this group were actively selected (e.g. Ephemerellidae) or randomly captured if they were highly abundant (Baetidae and Heptagenidae). These results agree with available information for the typical diet of this bird (Santamarina 1990; Tyler and Ormerod 1991; Bertrand 1994; Taylor and O’Halloran 2001; Horváth 2002). The selection by dippers of Brachycentridae and Dytiscidae, two groups that were avoided by *N. fodiens* and *G. pyrenaicus*, could be due to the ecology of this species. Brachycentridae individuals are small and are mainly found in the moss on rocks, usually in large quantities. Unlike the two mammals analysed in the stream, dippers' beaks will allow them to efficiently select these groups. Similarly, Dytiscidae beetles, swimmers found in areas of low flow (Tachet et al., 2010), are vulnerable to visual detection by dippers; the mammals, on the other hand, move amongst the benthic rocks of the stream and detect prey mainly using their vibrissae.

The diet of the water shrew *N. fodiens* in the Catllar stream was based on abundant trichopterans and ephemeropterans but also included terrestrial prey, which agrees with previous categorisations of this mammals as a generalist predator (Castién and Gosálbez 1999; Churchfield and Rychlik 2006). Even so, it also actively selected various families of dipterans and other groups found in low abundances. Its prey preference was focused on families that, on one hand, are easy to catch due to their low mobility and, on the other hand, are soft bodied and so completely and easily digestible. This fact would also explain the avoidance of and random feeding tendencies on families that are highly chitinous, have hard cases, or are difficult to find (Tachet et al. 2010; Biffi et al. 2017a). Most of the aquatic taxa consumed by *N. fodiens* were also preyed on by *G. pyrenaicus*.

4.2. Trophic ecology and diet specialization in *G. pyrenaicus*

The *G. pyrenaicus* population in the Catllar stream fed on the most abundant aquatic taxa of the stream (Baetidae, Nemouroidea, Hydropsychidae, Odontoceridae and Heptageniidae) but also selected low abundance families preyed upon by *N. fodiens* and *C. cinclus* (e.g. Tipulidae and Dixidae). Both mammals avoided the soft-bodied taxa that inhabit fine sediments (e.g. Athericidae, Limoniidae and Oligochaeta), a finding also reported in other studies of *G. pyrenaicus* (Esnaola et al. 2021). This situation illustrates the interspecific trophic competition occurring between *G. pyrenaicus* and the other analysed semiaquatic predators, as well as the partial prey overlap, in which these predators co-exist by frequenting a different microhabitat determined by their specific morphological and physiological adaptations (Castién and Gosálbez 1999; Churchfield and Rychlik 2006).

The Pyrenean desman is considered a generalist that feeds on the most abundant prey (Biffi et al. 2017a; Hawlitschek et al. 2018; Esnaola et al. 2021) and only a few studies have ever reported a specialist diet for this species

(Bertrand 1994). In our study, *G. pyrenaicus* focused on aquatic, energetically profitable prey. Its physiological and morphological adaptations to diving (Richard and Micheau 1975) explain its prey electivity in the Catllar stream, which is characterised by its choice of rheophile, benthic, low mobility, soft-bodied and abundant groups, but also its rejection of families with a low energetic yield (Bertrand et al. 1994; Biffi et al. 2017a) and avoidance of terrestrial prey. In this aquatic environment, the Pyrenean desman outcompetes both *N. fodiens* and *C. cinclus*. Although its avoidance of terrestrial prey observed in the Catllar stream matches other studies that have used a morphological approach for prey identification (Santamarina 1993; Bertrand, 1994; Castién and Gosálbez 1999), some studies based on a genetic approach (Biffi et al. 2017; Esnaola et al. 2021) indicate that the desman actively consumes terrestrial groups. Both these approaches permit the identification of a wide range of prey items but it is, nevertheless, important to understand the biology of these prey species when assessing their use as food resources in order to avoid misleading interpretations.

For instance, in many recent studies (Biffi et al. 2017a; Biffi et al. 2017b; Esnaola et al. 2021), the Diptera family Psychodidae has been reported to be an important component of the diet of the Pyrenean desman. In our study, individuals of this family were found in the analysed faecal samples but were also observed alive in faeces and later identified at the larval stage with no signs of having been digested. Given the biology of this family, we decided that this taxon should not be included as prey because the adults of many of its members lay their eggs in fresh depositions (Arnett 2000; Tachet et al. 2010). Thus, the finding of full-bodied, undigested individuals from this family does not indicate that they were used as a food resource. Hence, we believe it to be essential to collect as fresh as possible diet samples to avoid this kind of environmental contamination, and to undertake a morphological examination of samples to rule out any possible misinterpretation of results. In conjunction with the morphological identification of prey groups, genetic diet studies can confirm the importance of soft-bodied prey groups that are often missed by morphologic studies (Biffi et al. 2017a; Hawlitschek et al. 2018; Esnaola et al. 2021). For example, the molluscs Ancylidae and the Oligochaeta worms are difficult to detect morphologically after digestion due to their soft bodies, and their morphological identification is based on chaeta and shield parts that diminish their relative abundance in the diet of *G. pyrenaicus* and other stream predators.

Further research is still needed to fully understand prey electivity and diet specialisation in *G. pyrenaicus* and the role terrestrial prey items play therein. The benefits and limitations of both genetic and morphological approaches must be taken into account when designing studies aimed at assessing the diet of stream vertebrates such as *G. pyrenaicus* in order to avoid misleading interpretations.

4.3. Conservation of *G. pyrenaicus*: impacts of damming and a the need for action

The Pyrenean desman is restricted to productive, relatively pristine and well-preserved habitats in mountainous regions due to its sensibility to many anthropogenic stressors and its need for regular food availability throughout the year. Local population declines and extinctions have been reported in all the fluvial basins in its range. This has been attributed to the interaction of multiple anthropogenic stressors, of which those linked to hydrological alterations – above all, damming – are thought to be one of the main threats (Nores et al. 2007: Aymerich and Gosàlbez 2018; Esnaola et al. 2021).

Damming can directly impact the Pyrenean desman, on one hand, by impairing the connectivity between its populations (Allan and Castillo 2007; Nores et al. 2007) and, on the other, by causing a reduction in the water flow, and a decrease in the volume, area and depth of streams, thereby altering the velocity of its waters (Rolls et al. 2012). This reduces the heterogeneity of the stream habitat, as well as the availability of riffles, the fast-flowing sections of the stream that are a key requirement for the Pyrenean desman. They help it overcoming its natural buoyancy, allowing it to crawl down to the benthos and cling on with its strong claws as it feeds on its preferred prey (Nores 2007; Esnaola et al. 2021). The presence of a dam can also affect it indirectly by altering the availability of its food resources: the rheophile macroinvertebrate community of the stream. A reduction in the density and richness of rheophile prey species, especially those belonging to the Trichoptera, Plecoptera and Ephemeroptera orders, has been reported in many dam-impounded streams due to the negative effects these structures have on habitats (Lessard and Hayes 2001; Allan and Castillo 2007; Rolls et al. 2012; Martínez et al. 2013; Mor et al. 2018). Damming undoubtedly increases competition for their preferred resources, which is rheophile prey (Rolls et al. 2012).

A reduction in the density of stream macroinvertebrates caused by damming could, as a result of bottom-up processes (Livingston 1997; McIntosh et al. 2003; Hannah et al. 2007), intensify competitive trophic interactions between *G. pyrenaicus* and its competitors (Biffi et al. 2017a), thereby even further reducing the availability of resources for the Pyrenean desman's highly specialised diet. Considering that currently more than 115 hydropower dams exist in the Pyrenees (Durban 2011), this situation, along with other factors, could be contributing to current population declines in *G. pyrenaicus*, as its very specific diet could prove to be a disadvantage compared to other predators that feed on a wider variety of prey. This effect will not have such a strong effect on populations of either *C. cinclus*, which can easily migrate to other areas with better conditions and feed on terrestrial prey, or on populations of *N. fodiens*, which have higher individual densities and can also feed on terrestrial macroinvertebrates. This situation has been hinted at in the central Pyrenees, where *G. pyrenaicus* is experiencing severe population declines and is now found in only 45% of possible streams, whereas *N. fodiens* is found in 94% (Aymerich and Gosàlbez 2015). One possible solution to this problem is the complete removal of dams since the release of 'environmental flows' from dammed streams is not enough to preserve the populations of this endangered species (Esnaola et al. 2021). In fact, dam removal operations in the Basque Country (northern Iberian Peninsula) have improved the situation of the desman populations in affected streams (Urquijo 2018; Yarzabal et al. 2018; Esnaola et al. 2021).

More awareness is needed of the current situation of the Pyrenean desman. Due to the increase in average temperatures and reduction in water flow in the Pyrenees caused by climate change, the distribution of *G. pyrenaicus* is expected to severely decline during the present century (OPCC-CTP 2018). As a characteristic species of an emblematic and threatened habitat, *G. pyrenaicus* could be used as a flagship species for protecting whole ecosystems, and management actions could be focused on this single species. The European desman has been reported from 296 Natura 2000 sites and multiple European restoration projects have included actions in streams to improve the populations of this species. Despite this, its conservation is still being hindered by a lack of awareness in the human population of the situation of this threatened mammal (Hawlitschek et al. 2018; Esnaola et al. 2021). Bearing all this in mind, we believe that more research into how anthropogenic stressors affect the quality of suitable habitats for the

Pyrenean desman is urgently required and so we encourage scientists to instigate effective communication programmes aimed at raising awareness of this vulnerable species.

Chapter 4

Interacting impacts of damming and metal pollution on the endangered Pyrenean brook newt (*Calotriton asper*)

López-de Sancha, A., Vila-Gispert, A. and Guasch, H. (2022). Interacting impacts of damming and metal pollution on the endangered Pyrenean brook newt (*Calotriton asper*). Submitted to Herpetology Notes.

1. Introduction

Freshwater ecosystems are biodiversity hotspots that contain more than one third of all vertebrate species (Dudgeon et al., 2006; Bailan et al., 2008). One of the most threatened groups in these ecosystems are amphibians, with at least 40% of their species experiencing worldwide population declines (Bishop et al., 2012; Colomer et al., 2014). Amphibians depend on water quality and availability for their survival and reproduction, which makes them highly sensitive to threats like climate change (Walls et al., 2013), metal pollution (Dovick et al., 2020), stream regulation, and water-associated diseases, among others (Bednarek, 2001; Lessard and Hayes., 2003). These threats are especially relevant on mountain headwater streams, one of the most unique and vulnerable types of freshwater ecosystems. These environments are characterized by particular hydrological and morphological conditions, cold and oligosaline waters, and conspicuous seasonal variations in flow (Milner and Petts, 1994; Giller and Malmqvist, 1998; Maddock, 1999; Freeman et al., 2007). The morphology of these streams causes their waters to have a high energy that has historically been taken advantage of with the construction of hydroelectric dams (Zarfl et al., 2019) that impair the connectivity of streams and reduce their natural water flow, affecting their physical and chemical characteristics (Mor et al., 2018). In the recent decades, this type of hydromorphological alterations has been coupled with an increase on the metal and metalloid concentrations of their waters and sediments due to the proliferation of industries and mining (Colas et al., 2013), affecting many kinds of freshwater organisms due to their bioaccumulation in the trophic chain (Gessner and Tlili, 2016).

Field evidences of amphibian population declines due to metal and metalloid pollution are lacking (Chen et al., 2009; Gardner et al., 2018) and, in the current climate change context, the impacts of metal pollution on headwater streams could be exacerbated by the precipitation decrease that is expected in many mountainous regions. A good example to study these alterations is the Pyrenees mountain range, in the Mediterranean basin (north-west Iberian Peninsula). Climatic models predict on these mountains, by the end of the 21st century, a decrease in precipitations of between 10.7 and 14.8% and a mean increase in temperatures of between 2.8 and 4°C (López-Moreno et al., 2008a), as well as a decrease on the maximum accumulated snow level, on which streams depend on to maintain a natural and sufficient water flow (López-Moreno et al., 2009a). As a result, the water warming and flow reduction caused by climate change on headwater streams could interact with metal pollution and damming situations, threatening the water availability and quality that amphibians depend on in order to survive and reproduce, highlighting the need of analysing the impact of metal pollution on the populations of this sensitive group.

An emblematic and protected amphibian species found in Pyrenean headwater streams is the Pyrenean brook newt, *Calotriton asper* (Dugès, 1852). This newt species is an endemism found in the Pyrenees of Spain, France and Andorra, reaching the Pre-Pyrenees in some locations (Figure 1). It is a rheophile species, adapted to a benthic life, predominantly in cold and fast mountain streams with high slope, scarce vegetation, and a benthos dominated by stones and gravel. These newts prefer areas with enough shelter to hide from predators, such as under rocks and between vegetal debris (Montori et al., 2008; Montori and Llorente, 2014).

Figure 1. Current distribution of *Calotriton asper*, on the north-east of the Iberian Peninsula.

Pyrenean brook newts play an important role in the energy flow pathways of Pyrenean headwater streams as they can function as prey, competitors and predators in aquatic food webs. These amphibians predate on macroinvertebrate populations, driving top-down trophic cascades. Optimally, their diet consists of Plecoptera, Ephemeroptera, Trichoptera and

Diptera larvae. They can also act as generalists if needed, being able to consume other amphibians such as *Salamandra salamandra* in larval or metamorphic state, with which it can also present trophic competition, making it rare to found these two species in sympatry at the small scale (Montori, 1988; Sánchez-Hernández, 2020). At the same time, they suppose an important energy budget for higher trophic levels such as fish, snakes and mammals. The brown trout*, Salmo trutta fario* (Linnaeus, 1758), is the main predator of *C. asper*, but any medium-sized fish is a potential predator of this species (Montori, 1988). Predatory fish are a major force that structures amphibian assemblages as they can extirpate local populations, altering their distribution and abundance patterns (Vredenburg, 2004). In the southern slope of the Catalan Pyrenees, *C. asper* and *S. trutta fario* are found predominantly in an allopatric distribution, but some populations are sympatric with a spatial and trophic segregation (Montori, 1988; Montori et al., 2006). Moreover, the main dietary components of the brown trout are shared by the Pyrenean newt (Sánchez-Hernández et al., 2019), which may cause competition between these two species for basal resources. All these facts make *C. asper* a key species of the energy and nutrient flow pathways on Pyrenean headwater streams, and declines on its populations may drive negative consequences for the structure and function of these ecosystems (Davic and Welsh, 2004; Preston and Johnson, 2012; Sánchez-Hernández, 2020).

Population data for *C. asper* is scarce, and there is only one Pre-Pyrenean population that has been profoundly described, with an estimated density of between 3.500 and 5.500 individuals in a 1.5km long stream (Montori, 1988; Montori and Llorente, 2014). In the centre of its distribution area, this species should be frequent and abundant (Montori and Llorente, 2014), but in marginal areas it presents fragmented and less abundant populations, complicating the comparison between population densities found in the scientific literature. For example, densities in bordering areas of the Basque Country (Northern Spain) are estimated to be between 17,71 and 72 individuals/km (Gosá and Bergerandi, 1994; Arrayago et al., 2005).

The major threats to this species are the loss and degradation of aquatic habitats, hydrological alteration through dams and weirs, infrastructure development of skiing stations, diseases, pesticide and metal pollution, salmonid introductions and climate change (Montori et al., 2002; Daszak et al., 2003; Bosch et al., 2008; Colomer et al., 2014). This species is listed by the

IUCN Red List as Near Threatened (NT) because its area of distribution is less than 20.000km^2 and its suitable habitat is declining (Bosch et al., 2008). It is also included under the category of Special Interest in the National Catalogue of Endangered Species (Bosch et al, 2008), and the IUCN Red List recognises the need of updating the threat categorization for this species, since last assessment was done more than 12 years ago. The Spanish population of this species is also considered Near Threatened (Montori et al., 2002) and is protected by the Spanish Royal Decree of 1980 and 1986, being included in the Berna agreement annex and in the II and IV annexes of the EU Habitats Directive. In this regard, new research is required to know current population sizes, distributions and trends of this species, as well as the analysis of their threats and actions needed to improve its conservation (Bosch et al, 2008).

Despite amphibians being so sensitive to hydrological alterations, and considering that the hydrological regime plays a significant role in the structuring of amphibian communities, the impacts of stream regulation on amphibians has barely been assessed (Wassens and Maher, 2011). Damming threatens stream ecosystems by causing habitat degradation and fragmentation, flow reduction, changes in the sediment transport, water temperature increase, deterioration in water quality, biodiversity loss and spreading of water-associated diseases (Bednarek, 2001; Lessard and Hayes., 2003).

Another important threat to amphibians, including *C. asper*, is chytridiomycosis, a wildlife emerging disease caused by the pathogenic fungi *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium salamandrivorans* (Bsal), which have already caused significant declines of amphibian populations around the world, including different areas of Spain, with cases on *C. asper* reported on Catalonia and the Pyrenees (Martínez-Silvestre et al., 2020). *C. asper*, being stream breeders, having a low fecundity and being habitat specialists in headwater streams, are really susceptible to these fungi, as they have a faster growth in cool temperatures, have a wide host range, high virulence and asymptomatic infection in larvae. Even more, they have the potential ability to survive outside the host. Awareness of infectious diseases as a threat to wildlife, especially to amphibians, has raised in recent years but, generally, they remain poorly studied and understood (Duffus and Cunningham, 2010). Field monitoring of wild populations, including asymptomatic individuals, coupled with management practices, must be performed and improved in order to understand the distribution of this disease and prevent its further spreading (Athan et al., 2005; Martínez-Silvestre et al., 2020).

In order to conserve amphibian populations, we need to characterize them, assess their threats, and project future plans for their management, considering that the scale at which these actions are performed is crucial. *Calotriton asper*, being a Pyrenean endemism, presents a small distribution, and the management of small headwater streams could be decisive for the survival of its populations (Montori et al., 2007). Because habitat alteration and degradation are potentially reversible, defining the critical factors needed for restoring and conserving high-quality habitats for amphibians is essential if we aim to maintain the ecosystem structure in headwater streams (Semlitsch, 2002).

Within this context, our study focuses on a small Pyrenean headwater stream affected by a dam and by metal and metalloid pollution. The main objective of this study is to perform a case study in which we will characterise the population of *C. asper* in this stream and understand how damming, metal pollution, and the potential prevalence of chytridiomycosis cause differences in the population density and body size between subpopulations at each side of the dam. We will consider the management and conservation needs that these impacts might suppose in order to sustain the correct ecosystem function structure of headwater streams with amphibians. The population of *Salmo trutta fario* of the stream will also be characterised in order to consider the predator pressure on this *C. asper* population. Information about the water characteristics, the metal and metalloid concentration, and the macroinvertebrate availability as food resources of the analysed stream will be used from the previous chapters of this thesis. We hypothesize that the differences in the metal and metalloid concentrations of the water at each side of the dam, caused by the water diversion that the dam implies, will cause differences between subpopulations in the population density and body sizes of *C. asper*.

2. Methodology

2.1. Study Area

The study area was a small Pyrenean headwater stream named Catllar, an influent of the Ter River, in Vilallonga del Ter, Catalonia (NE Iberian Peninsula), located between 1200 and 1600 m.a.s.l. A hydroelectric dam divides the stream, deviating most of the upstream water to a hydroelectric central, so downstream water comes mainly from the adjacent west stream. We selected two sampling transects of 500m each at both sides of the dam. An old, abandoned Sb mine that leaks metals and metalloids was present west to the highest part of the upstream transect (Figure 2).

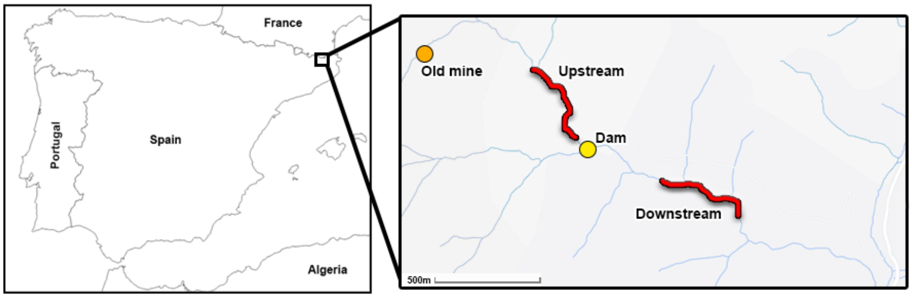

Figure 2. Catllar stream basin with its main tributaries, indicating the two sampled transects with red lines (Upstream and Downstream), the dam with a yellow dot, and the abandoned mine with an orange dot. Catllar stream is a tributary of the Ter River located in Catalonia, NE Iberian Peninsula (black box).

2.2. Habitat characterization and newt samplings

Transects were characterized previously to the newt samplings by identifying all the potential habitats and refugia for the newts following the characterization methodology proposed by Montori et al. (2008). Newt samplings were performed on summer 2021, between July and August, sampling each transect from 21:00PM to 03:00AM approximately, time when newts are the most active (Hervant et al., 2000). The same sampling effort was maintained for each transect. Transects were sampled counter-current in order to avoid generating water turbidity, with an active visual search of individuals on the riverbed and in the typical *C. asper* habitats, with the help of a small hand net and a regular light torch. Two samplings on each transect were performed in consecutive nights in order to estimate their subpopulation density using the two-event mark-recapture methodology proposed by Chapman (1951), considering the first sampling as capture, the second as recapture, and assessing which individuals from the second sampling were previously found on the first. Due to the low density of individuals in the stream, the sampling effort was doubled, performing two samplings for each capture and two samplings for each recapture at each transect. Individuals from both samplings of the same capture or recapture event were grouped and counted as a single capture, but if an individual was found at both samplings of a single event, it was not considered for the calculations. Captured individuals were measured using the total length (TL) and the snout-to-vent length (SVL) (Trochet et al., 2019), weighted, sexed, and a picture from their ventral area was taken to individually identify them in order to assess the recaptures. Captured individuals were returned safely to the spot where they were found.

If salamander (*Salamandra salamandra*) larvae were found during the newt samplings, they were also counted in order to assess the cooccurrence of both species and its possible implications.

2.3. Chytridiomycosis detection

Captured individuals were scrapped using cotton swabs following a standardized protocol: they were swabbed 15 times in the abdominal area and 15 more times divided between the fingers of all four legs (Figure 3). Swabs were frozen at -20ºC until their analysis for the detection of *B. dendrobatidis* and *B. salamandrivorans* following the PCR protocol described in Blooi et al. (2013).

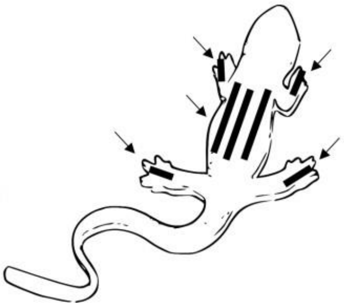

Figure 3. Swabbing areas sampled in *C. asper* for the detection of *Bd* and *Bsal* spores.

2.4. Fish community sampling

In order to assess the predation pressure on the newt, we characterised the fish community of the stream using electrofishing gear (Electracatch WFC4 - High Voltage pulsed DC electrofishing system (Electracatch International, Wolverhampton, UK)) to sample at both sides of the dam on July, previously to the newt samplings. The IBICAT methodology (ACA, 2010) was followed respecting the standardized European normative (CEN, 2003). A 100m-long transect, including all the stream width, was delimited with fishing nets and 3 successive catches were performed, removing all the fishes captured to avoid recaptures. All fishes were weighted and measured considering the standard length, from the tip of the snout to the posterior end of the last vertebra.

2.5. Ethics and biosecurity

Capture permits for *C. asper* and *S. trutta fario* were granted by the Catalan government and biosecurity measures were ensured during all the samplings. A new pair of gloves was used for the handling of each individual and boots and hand nets were cleaned after each sampling using a water and bleach solution in order to avoid spreading possible *Bd* and *Bsal* spores and other potential diseases. All the individuals were returned safely to the point where they were found.

2.6. Statistical analysis

All statistical analyses were performed using the RStudio software version 1.2.5033 (RStudio Team, 2019). *C. asper* population density estimations and the standard error of the estimator for each transect were obtained by using the nChapman and the seChapman functions in the R package *recapr,* version 0.4.3. In order to check for differences in the density of individuals between positions from the dam, the ciChapman function of the package was used in order to obtain 95% confidence intervals of the estimated population density by bootstrapping and by using a normal distribution. Weight and length (TL and SVL) measurements of *C. asper* were checked for differences between subpopulations at each side of the dam using a t-test. The lineal regression between those size variables was assessed to check for slope homogeneity by applying an ANCOVA analysis to the model of the length and weight relation plus their interaction, using the position from the dam as a factor. Afterwards, the same ANCOVA analysis was applied without considering the interaction in order to assess the differences in the physiological condition of the newts between positions.

Fish population density was estimated using the Carle-Strub method in the *Simple Fisheries Stock Assessment Methods* R package (Ogle et al., 2019), based on the maximum likelihood estimation of population size from removal data (Carle and Strub, 1978). Length and weight classes of the captured fishes were established and compared using a Chi-Square test in order to assess differences on fish sizes between subpopulations at each side of the dam.

Stream water characteristics and metal and metalloid concentration data from the Chapter 1 of this thesis, and macroinvertebrate community data from Chapter 2 was used for the interpretation of the results of this study.

3. Results

3.1. Habitat characterization

The predominant habitat type found in the stream were refugia (big rocks, caves, roots, bushes and logs), pools and waterfalls (Table 1). This stream had abundant suitable habitats for *C. asper*, with a similar total number between positions, but with differences in the distribution: more refugia were found downstream, but more pools, waterfalls and wells were found upstream.

Table 1. Counts for the potential habitats of *C. asper* found in the characterization of the Catllar stream, grouped by relative position from the dam.

Transect	Pools	Waterfalls	Refugia	Wells	Total habitats	Average width (m)	Average depth (m)
Upstream	78	73	230	19	400	3.59 ± 0 .58	0.31 ± 0.05
Downstream	66	58	293	11	428	4.20 ± 1.06	0.33 ± 0.06

3.2. *C. asper* population and *S. salamandra* presence

The downstream transect presented a significantly higher population density of *C. asper*, without an overlapping of the confidence intervals for their estimations (Table 2). The body size analyses showed that the downstream subpopulation had a higher weight (t= 4.35, P<0.001) and a higher TL (t= 4.05, P<0.001) and SVL (t= 4.93, P<0.001) than the upstream subpopulation. The ANCOVA analyses showed that there was a lineal regression between their length and weight, with slope homogeneity, and without a significant interaction nor differences between positions (Supplementary material: Table 1 and Table 2). Consequently, both subpopulations at each side of the dam had similar physiological conditions, but upstream newts were smaller than downstream ones. *C. asper* individuals with deformities were found at each side of the dam: 15.25% of the upstream subpopulation and 10.71% of the downstream subpopulation presented deformities (Figure 4).

S. salamandra larval individuals were predominantly found at the upstream transect, with 27 individuals grouped in small pools, while only 3 larvae were found downstream.

Table 2. Captured individuals for each capture-recapture event and estimated population density of *C. asper* at each side of the dam, with the body size characteristics of each subpopulation. Values present the standard error of the averaged value. Confidence intervals for the population density estimates are present with the normal and the bootstrap estimations**.**

Parameters	Upstream	Downstream
1st sampling	16	32
2nd sampling	18	32
Recaptures	6	5
Estimated density (ind/500m)	45.1 ± 9.9	180.5 ± 56.1
95% C.I. (Normal)	[25.7 – 64.6]	[70.5 – 290.5]
95% C.I. (Bootstrap)	[28.4 – 106.7]	[107.9 – 543.5]
Avg. weight (g)	10.7 ± 0.5	13.2 ± 0.3
Avg. TL (mm)	127.8 ± 1.7	137.3 ± 1.6
Avg. SVL (mm)	70.4 ± 1.0	77.1 ± 0.8

Figure 4. Deformed leg of an individual of the upstream subpopulation of *C. asper*, showing its front leg composed exclusively by one finger.

3.3. Chytridiomycosis

The results of the PCR indicated that there was no presence of *B. dendrobatidis* nor of *B. salamandrivorans* in the individuals of *Calotriton asper* that were sampled in the Catllar stream.

3.4. Fish community

A single fish species was found during the fish samplings: *Salmo trutta fario* (Linnaeus, 1758). Estimated individual densities between subpopulations of this species at each side of the dam did not present statistically significant differences. The estimated biomass was slightly higher downstream (Table 3) but, despite this, once classified by weight and length classes (Supplementary material: Table 3 and Table 4), no significant differences in sizes between subpopulations were found between positions (Table 4).

Table 3. Captured trouts in the Catllar stream and its estimated (est.) population density, with averaged values of standard length and weight, including the standard error. Also, estimated trout density and biomass per hectare considering the average transect width.

Position	Captured trouts	Est. density (ind/100m)	Avg. standard length (cm)	Average weight (g)	Est. density (ind/ha)	Est. biomass (kg/ha)	Average transects width (m)
Upstream	30	33 ± 2.57	9.25 ± 4.57	22.13 ± 26.74	866 ± 70.41	0.57	3.65
Downstream	24	32 ± 4.81	9.02 ± 3.65	17.71 ± 14.33	876 ± 126.25	0.73	3.81

Table 4. Chi-Square test assessing statistical differences between positions from the dam by weight and length classes for the *Salmo trutta fario* subpopulations.

	X-squared	Degrees of freedom	p-value
Weight classes	6.938	7	0.435
Standard length classes	3.999	6	0.677

3.5. Environmental characterization

The previous study of the biotic and abiotic characteristics of this stream showed that the downstream transect had a lower water flow with a higher conductivity and pH. Also, being marginally statistically significant (P=0.06), downstream water was warmer than upstream. No differences in macroinvertebrate biomass and diversity were found between positions (Supplementary material: Table 5), and the biomass of the Ephemeroptera, Plecoptera, Trichoptera and Diptera groups did not differ between positions either (Supplementary material: Table 6). Metal and metalloid concentrations in water and in biofilms were found to be higher upstream from the dam for As in water and of Ni, Cu and As in biofilms (Supplementary material: Table 7), showcasing the differences in the metal impacts on the trophic chain at each side of the dam.

4. Discussion

The hereby studied population of *Calotriton asper* is the first record of this species, and the first description of its status, on the Catllar stream, for the Vilallonga del Ter municipality, but its presence has been cited before in the 10x10km grid where the stream is found (Villares and Ruiz, 2020). This population is located on the southern slope of the central distribution area for this species, and it is found in a habitat type with suitable conditions for it (Montori et al., 2008; Montori and Llorente, 2014). If abiotic conditions are favourable and food resources are abundant, the main driver of population density for *C. asper* is the availability of suitable habitat and refugia (Montori et al., 2008). As seen in the habitat characterization of this study, and in the previous environmental characterization, this stream presents favourable conditions for this species at both sides of the dam: a headwater stream with high slope and low water temperature, abundant and variable habitats and refugia, low vegetation at its margins and a benthos dominated by rocks and gravel (Montori and Llorente, 2014). Therefore, a high population density of *C. asper* should be expected but, instead, the population densities found were similar to the ones present in the bordering areas of its distribution (Gosá and Bergerandi, 1994; Arrayago et al., 2005).

Differences were also observed between subpopulations at each side of the dam, with a higher density downstream. It is known that *C. asper* is a sedentary species and has a constant distribution, with yearly longitudinal movements of less than 50m, and their population density remains fairly constant throughout the year (Montori et al., 2008; Montori et al., 2012). Moreover, the newts from the upstream subpopulation were smaller, although the physiological condition of both subpopulations did not differ between positions. Consequently, the Catllar population might be affected by adverse biotic or abiotic conditions that is causing population density and body size differences between subpopulations at each side of the dam.

4.1. Biotic interactions of *C. asper* with *S. salamandra* and *Salmo trutta fario*

Biotic interactions such as competition and predation could be causing these differences between subpopulations at each side of the dam, but it does not seem to be the case. On one hand, *S. salamandra* and *C. asper* are trophic competitors and present a coincidental spatial distribution, but are rarely found in sympatry at the small scale due to the predation of *C. asper* larvae over the larvae of *S. salamandra* and the dominance of *C. asper* over the competition for trophic resources (Montori, 1988). In our study, only three larval individuals of *S. salamandra* were found downstream from the dam, where the density of *C. asper* was much higher, while their presence was higher upstream, where a lower density of *C. asper* was found. These results are also coincident with other studies (Arrayago et al., 2005; Guillaume, 2006) and show a displacement from *C. asper* over *S. salamandra* in this stream and, consequently, a lack of interspecific competition between them. This fact also supports the accuracy of the *C. asper* population density estimates in the Catllar stream, as the upstream higher presence of *Salamandra* would be favoured by the low *C. asper* density.

On the other hand, the brown trout, *Salmo trutta fario*, is the main predator of *C. asper*. The brown trout is usually found in allopatry with the Pyrenean newt in the southern slope of the Catalan Pyrenees, and in populations where they are sympatric, like in the Catllar stream, they present a spatial and trophic segregation (Montori, 1988). Despite this, newts are usually a low fraction in the stomach content of trouts, being predated only occasionally. Trouts and newts also compete for the same trophic resources (Hartel et al., 2007; Montori and Llorente, 2014). Normal estimated abundances of this trout species on Pyrenean streams are between 0,355 ind/m^2 and 0,898 ind/m^2 (García de Jalón et al., 1986), which indicates that the trout population in the Catllar stream is also underrepresented, with an estimated density of around 0,08 ind/m^2. Trout subpopulations at each side of the dam presented similar population densities and sizes, which hints that they exert a small and similar predation pressure over both *C. asper* subpopulations. Consequently, the trout presence might be a factor that contributes to the overall low newt density of the stream, especially considering the fact that this stream has a high water flow on autumn, due to the increase in precipitations, and during spring and early summer, due to the snowmelt, allowing the trouts to reach shallow spots of the stream that would be used by newts to reproduce during those seasons. Despite this, as a similar trout density was found at both sides of the dam, the observed differences between the subpopulations of *C. asper* should be mainly driven by other factors than predation and competition, and the availability of trophic resources must be considered.

4.2. Food resources for *C. asper* in the Catllar stream

The diet of *C. asper* is primarily based on aquatic macroinvertebrates, mainly Plecoptera, Ephemeroptera, Trichoptera and Diptera, with less than 10% of their diet based on terrestrial prey (Montori and Llorente, 2014). This species presents differences on its diet during different life stages. While adults are generalists and feed on small-sized macroinvertebrates, larval newts focus on Diptera and Ephemeroptera larvae (Montori, 1991; Montori and Llorente, 2014). As we observed in the previous environmental characterization, Ephemeroptera, Plecoptera and Trichoptera, as well as Chironomidae (the main Diptera taxon found in the stream), were highly abundant groups, and their biomass and the EPT biodiversity index did not differ between positions from the dam. Consequently, *C. asper* did not lack food resources as larvae nor as adults in this stream, and its availability did not differ at each side of the dam. This was confirmed by the lack of differences in the physiological condition of both subpopulations at each side of the dam. In consequence, it seems that prey availability was not a limiting factor either for *C. asper* in this stream, and the low population densities and the differences between upstream and downstream subpopulations in this stream might be caused by abiotic factors.

4.3. Metal and metalloid pollution diminishes the population density and the body size of *C. asper*

One of the most feasible explanations for the overall low density of *C. asper* in the Catllar stream and the disparity of population densities and body sizes between positions is the metal and metalloid pollution situation of the stream. Metal pollution is considered one of the major drivers of worldwide amphibian populations decline (Blaustein et al., 2003). Amphibians are useful bioindicators of pollution due to their toxin absorption through respiration (Dovick et al., 2020) and sediment ingestion gulped during predation (Adlassnig et al., 2013). In the Catllar stream, there was a significantly higher concentration of As in water, and of As, Ni and Cu in biofilms, upstream from the dam due to the water diversion that it implies. Other metals such as Cr, Zn, Cd and Sb had also been found all along the stream. All these metals and metalloids have been proven to cause negative effects in many amphibian species such as deformities, delayed development and reduced size, changes in antipredator behaviour and reduction in fitness and survival (Nebeker et al., 1995; Chen et al., 2009; Gay et al., 2013; Adlassnig et al., 2013; Dovick et al., 2020).

Gardner et al. (2018) studied how the environmental exposure to As and Cr affected another species of salamander (*Ambystoma gracile* (Baird, 1857)), and they proved that water concentrations of at least 5.99 µg/L for As and 1.45 µg/L of Cr caused the bioaccumulation of these metals in their organs, altering their functioning and causing DNA damage. Moreover, both metals are reported to being transferred from parents to offspring (Magari et al., 2002; Gardner et al., 2018). Levels of Cr in the Catllar stream were similar to the ones found in this mentioned study, and As levels were much higher, especially upstream from the dam. *Calotriton asper* could be affected in the same way as *A. gracile* by this process, considering that it belongs to the same family as the salamander used on that study and that it is a species that spends most of its life cycle inside the water (Montori, 1988), thus being even more exposed to the pollution. In our study, we also found many individuals with deformities on both subpopulations, a phenomenon that has been reported in cases of metal and metalloid pollution (Adlassnig et al., 2013) and should hint the bioaccumulation of these metals on *C. asper*. Upstream newts were smaller than the downstream subpopulation, and considering the previously mentioned effects of metals on amphibian size, development and survival, and the lack of differences between positions for food resources and predator pressure, metal pollution should be one of the main causes for a decrease in population density and body size on *C. asper*, causing the differences between positions from the dam that we observe.

It could be discussed that size differences between subpopulations are caused by temperature differences, because the size of ectothermic species is dependent on water temperature, but it is known that *C. asper* populations from higher altitudes and colder waters are larger than ones from lower altitudes (Trochet et al., 2019), and in the previous characterization of this stream we found warmer temperatures below the dam. Predation pressure also affects body size (Diego-Rasilla, 2003), but trout density and sizes was similar at both positions. Also, it could be argued that the upstream subpopulation is younger than the downstream one, but this species is long-lived and sedentary (Montori and Llorente, 2014) and only adult individuals were found, so these are improbable explanations and the metal pollution hypothesis seems far more feasible. However, it is also possible that the metal pollution situation in the upstream reach is reducing the survival of the subpopulation (Adlassnig et al., 2013), and consequently, this subpopulation is smaller due to it being younger.

4.4. Damming impacts on the Catllar *C. asper* population

It is known that stream regulation has a profound impact on the hydrological regime and connectivity of streams, causing habitat fragmentation (Bednarek, 2001). Migration in *C. asper* is key for the colonization of new habitats, to offset the passive downstream drift and to re-colonize habitats after floods (Montori et al., 2008), so the presence of a dam in the Catllar stream might be another main cause for the low overall newt population density. This dam supposes an impassable barrier for *C. asper*, inside and outside the stream, which may be leading to fitness losses due to inbreeding, and even to local extinctions. This kind of threat is stronger for small-sized populations, such as the Catllar one (Montori et al., 2007; Miró et al., 2018). Moreover, the water diversion that the Catllar dam implies also reduces the downstream water concentration of metals and metalloids, improving the water quality on that reach, causing the disparity that we observe between subpopulations, allowing for a higher population density and body size of *C. asper* downstream from the dam.

4.5. Chytridiomycosis on hydrologically altered Pyrenean streams

The Catllar population of *C. asper* was not affected by chytridiomycosis, but it is susceptible to be affected by this disease. The most recent review on the prevalence of *Bd* and *Bsal* on the Pyrenees (Martínez-Silvestre et al., 2020) indicates that *Bsal* has still not been reported in any Pyrenean population of *C. asper*, and that *Bd* was found only in 5 of the Spanish populations from the 29 French, Andorran and Spanish populations that they sampled. One of these affected populations was found at the locality of Camprodon, which is located only 8km away from our studied population, making the Catllar stream population susceptible to the infection. The susceptibility to this disease depends also on the water temperature (Walker et al., 2010; Clare et al., 2016; Greenspan et al., 2017). Consequently, the predicted warming of Pyrenean waters due to climate change, and the water flow reduction and temperature increase that damming causes, could furtherly increase the risk of chytridiomycosis on Pyrenean streams affected by damming.

4.6. Research and management of small populations of *C. asper* is crucial for its conservation

C. asper is not only a flagship species of the Pyrenees, but also a key structural component of the correct functioning of Pyrenean headwater streams. It has an important role on trophic top-down control, it is a food resource for higher trophic levels, and it supposes a link between aquatic and terrestrial environments (Sánchez-Hernández, 2020). Assessing and managing the state of small populations, such as the one in the Catllar stream, might not be relevant for the survival of the whole species, but it is fundamental for the local conservation of bigger populations (Montori et al., 2007). The conservation status of this species needs to be reassessed, and new population data is required (Bosch et al, 2008). Studying small populations of *C. asper* should be promoted not only to increase the availability of population data and assess the actual conservation status of this species, but also to understand how anthropogenic threats that are found in many streams along its distribution area may impair its survival. Monitoring wildlife populations is essential in order to increase the effectivity of conservation measures (Badia-Boher et al., 2021), and case studies like this one provide new information about the role of *C. asper* as bioindicator in a realistic and applied context, indicating how this species can be affected by current and rising threats like metal pollution and damming, providing relevant and applied knowledge to propose management actuations that could be effective for the conservation of the headwater streams that *C. asper* depends on.

5. Conclusions

In light of the previously reported effects of metals and metalloids on the size and development of newts and salamanders, the connectivity loss derived from the presence of the dam, and considering all the other biotic and abiotic parameters analysed in this stream, it could be argued that the most feasible explanation for the low individual density of *C. asper* in the Catllar stream population is caused mainly by the combined effect of the trout presence, the dam-derived impacts and the metal pollution situation. The differences in population density and body size between subpopulations at each side of the dam would be caused mainly by the water diversion that the dam implies, as it would, on one hand, reduce the metal concentrations downstream and, on the other hand, avoid the migration of individuals between subpopulations. Our case study provides an integrative approach on how the interaction of metal pollution and damming can affect small populations of this emblematic and endangered amphibian species. This study also provides new information on the prevalence of chytridiomycosis in the Pyrenees by describing a non-infected population that is close to an infected one while considering its implications in a context of applied conservation ecology.

6. Supplementary material

Table 1. ANCOVA results of the lineal regression analyses of slope homogeneity checks for *C. asper* TL and SVL in relation to weight, including their interaction, using the relative position to the dam as the factor. Degrees of freedom are 1 for all the tests.

	Sum. Square	**F**	**p-value**
SVL	246.11	101.07	**<0.001**
Position (SVL)	2.57	1.05	0.30
SVL*Position	1.89	0.78	0.38
TL	268.09	112.69	**<0.001**
Position (TL)	0.18	0.08	0.78
TL*Position	0.26	0.11	0.74

Table 2. ANCOVA results of *C. asper* TL and SVL in relation to weight in order to assess the condition of the newts between positions. Degrees of freedom are 1 for all the tests.

	Sum. Square	**F**	**p-value**
SVL	340.09	140.05	**<0.001**
Position (SVL)	8.12	3.34	0.07
TL	346.36	147.15	**<0.001**
Position (TL)	0.92	0.39	0.54

Table 3. River trout individuals found of each weight class (in grams) at each position from the dam.

Position	**0-0.9**	**1-8.4**	**8.5-15.9**	**16-23.4**	**23.5-30.9**	**31-38.4**	**38.5-45.9**	**>46**
Upstream	7	2	10	3	2	1	0	4
Downstream	4	3	7	1	4	2	2	1

Table 4. River trout individuals found of each length class (in centimetres) at each position from the dam.

Position	**0-2.9**	**3-5.4**	**5.5-7.9**	**8-10.4**	**10.5-12.9**	**13-15.5**	**>15.5**
Upstream	4	3	3	11	4	2	3
Downstream	3	2	2	8	6	3	0

General discussion

Biodiversity conservation is key for the sustainability of ecosystems in a context of environmental change. These changes can occur naturally, but the growing anthropogenic pressure on the biosphere is impacting the structure and function of most ecosystems and impairing the services that they provide (Loreau and Mazancourt, 2013). One of these ecosystems are headwater streams, hotspots of biodiversity that are particularly sensitive to environmental changes, especially to those concerning water flow alterations (Sabater and Tockner, 2009). This kind of alterations are linked to changes in their biotic communities and, because many streams have been intensely used by humans since ancient times, very few of them currently maintain their original ecological integrity due to the impacts of river regulation, channelization, pollution (Maddock, 1999; Alexandre and Almeida, 2009) and climate change (Daufresne et al., 2009), among others.

The ecological condition, water quality and environmental health of streams are frequently assessed using biotic and abiotic components of the ecosystem (Hornback et al., 2016). Structural components of the ecosystems, such as different biodiversity or water quality parameters, respond differently to many kinds of stressors which, at the same time, could interact among them exacerbating or diminishing their individual effects on the ecosystem. Understanding the response of the structural components of the ecosystem to multiple stressors improves our knowledge on the consequences of environmental change (Gessner and Tlili, 2016). In this regard, assessing the responses to those stressors in headwater streams is fundamental for their sustainable management and protection (Hornback et al., 2016).

The main results of this thesis show, on one hand, that the use of several taxonomic groups from different trophic levels as bioindicators, provide diverse and complementary information in the assessment of the environmental health of headwater streams. On the other hand, that the biotic communities of these ecosystems do not present homogeneous responses in front of different anthropogenic stressors. It was assessed how the hydrologic regime of a Pyrenean headwater stream was modulated by seasonality, the precipitation regime, the presence of a dam, and the interaction between them (**Chapter 1**), and how water flow and metal pollution in the stream affect its biotic communities (**Chapters 1**, **2** and **4**). We also assessed the role that macroinvertebrates play as trophic resources for stream vertebrates, and the consequences of the resulting trophic competition for them on the endangered Pyrenean desman, *Galemys* *pyrenaicus* (**Chapter 3**). Finally, we assessed how the previously analysed stressors of this stream affected a population of the Pyrenean brook newt, *Calotriton asper*, discussing the management actions that should be promoted to maintain healthy populations of this species in Pyrenean streams with similar conditions (**Chapter 4**). Considering the results of this thesis, in a climate change context, we consider that urgent and effective management actions in Pyrenean headwater streams affected by damming and pollution are needed in order to prevent further alterations of the ecosystem structure and function.

These main results are discussed in the following parts throughout this section, considering an applied conservation and management context: **i)** bioindicators for a multi-metric assessment of anthropogenic impacts on Pyrenean headwater streams; **ii)** climate change impacts on the ecosystem structure and function of Pyrenean headwater streams; **iii)** damming impacts on the ecosystem structure and function of Pyrenean headwater streams; **iv)** ecotoxicology of hydrologically altered Pyrenean headwater streams; **v)** conservation and management of Pyrenean headwater streams; **vi)** research needs, future perspectives and contributions of this thesis.

1. Bioindicators for a multi-metric assessment of anthropogenic impacts on Pyrenean headwater streams

Historically, the main bioindicator groups used to assess the impacts of anthropogenic disturbances in rivers and streams have been biofilms, macroinvertebrates and fish, but also, to a lesser extent, macrophytes and amphibians. As discussed in the review by Marzin et al. (2012), the responses of these groups to environmental stressors are usually assessed separately, and only few authors have integrated these responses under a multiple stressor situation, as this thesis presents. This kind of studies show that the response patterns and robustness of the structural metrics analysed differ considerably among bioindicators and stressor type. The results of this thesis support this claim, and integrates it in a context of headwater streams that are affected by common and interacting anthropogenic pressures.

The major changes observed on the biotic communities of the Catllar stream were mainly driven by seasonality, the precipitation regime and metal pollution, with a weaker influence of damming, which had a main role in the diversion of polluted water. These findings support the general consideration that bioindicator responses are stronger for water quality than for hydromorphological alterations (Hering et al., 2006). The weak responses of the analysed metrics to damming could be attributed to the adaptations of Mediterranean biota to a naturally high seasonal and interannual flow variability, allowing these organisms to follow those seasonal dynamics and to be less sensitive to flow reduction (Sabater and Tockner, 2009).

Organisms in communities inhabiting Pyrenean headwater streams are especially sensitive to environmental change, as they are adapted to relatively narrow ranges of abiotic conditions due to the multiple microhabitats that mountain streams generate (Bona et al., 2008). Therefore, their presence or absence, and its state of health, provides valuable information about the status of the ecosystem (OPCC-CTP, 2018). In this thesis, we analysed how multiple anthropogenic stressors affected several ecosystem metrics, assessed for each bioindicator group (Table 1). Moreover, we analysed the trophic interactions of predation and competence between those groups (Figure 1). Overall, the results obtained in this thesis highlight the value of using a large set of response variables in a multi-metric assessment with different bioindicator groups to obtain local, as well as general, information about the environmental health of Pyrenean headwater streams (Hopkins et al., 2011). Macroinvertebrate and biofilm metrics were especially useful for this purpose, as they presented multiple responses to environmental parameters that were reflected on both the structure and the function of their communities **(Chapters 1** and **2)**.

The multi-metric assessment with different bioindicator groups suggested that the response of biotic communities to multiple anthropogenic stressors on headwater streams may be better analysed if measures of both ecosystem structure and function are simultaneously taken, as they provide complementary, not redundant information (Hopkins et al., 2011). Research on how biodiversity changes affect the ecosystem function of freshwater ecosystems is increasing, but there is still a huge gap in the knowledge about the properties of the relationship between biodiversity and ecosystem function, and how anthropogenic impacts modulate it (Dudgeon, 2010; Hopkins et al., 2011). In this regard, the analyses using macroinvertebrate functional feeding groups (**Chapter 2**) showed that this approach is effective in the assessment of this biodiversity-ecosystem function relationship, without requiring an additional sampling effort. Because of this, its use is encouraged for the management and applied research of headwater streams.

Table 1. Summary of the metrics used for each bioindicator to assess the impacts of environmental factors (seasonality and precipitation regime) and anthropogenic stressors (damming and metal pollution) on the ecosystem structure and function of the Catllar stream. The assessed metrics are grouped into categories: structural (**S**) or functional (**F**) measures of the ecosystem. Coloured squares indicate an effect of a parameter on that metric. White squares indicate that the effect of a parameter on that metric was not assessed or that not enough evidence was observed to indicate an effect.

Bioindicator	Metric assessed	Seasonality	Precipitation regime	Damming	Metal pollution
Biofilm community	**(S)** Biomass	🟩	🟩	🟩	
	(S) Biodiversity	🟩	🟩	🟩	
	(F) Autotrophic Index	🟩	🟩	🟩	
	(F) Organic matter content			🟩	
Macroinvertebrate community	**(S)** Biomass	🟧		🟧	🟧
	(S) Abundance	🟧			🟧
	(S) Biodiversity	🟧	🟧	🟧	🟧
	(S + F) Functional Feeding Groups	🟧	🟧	🟧	🟧
Calotriton asper	**(S)** Population density			🟨	🟨
	(S) Individual size			🟨	🟨
Salmo trutta fario	**(S)** Population density				
	(S) Size classes				

This thesis also provided field evidences of the impacts of damming and metal pollution on the ecosystem structure by using as a bioindicator a population of the Pyrenean brook newt, *Calotriton asper* (**Chapter 4**). It is known that amphibians are good water quality and availability indicators, but the impacts of damming and metal pollution on this group have rarely been assessed (Chen et al., 2009; Reich et al., 2009; Wassens and Maher, 2011; Gardner et al., 2018). Detecting low densities of *C. asper* on areas where they should be abundant, and observing many small individuals with deformities, could indicate the impacts of damming and metal pollution on that ecosystem.

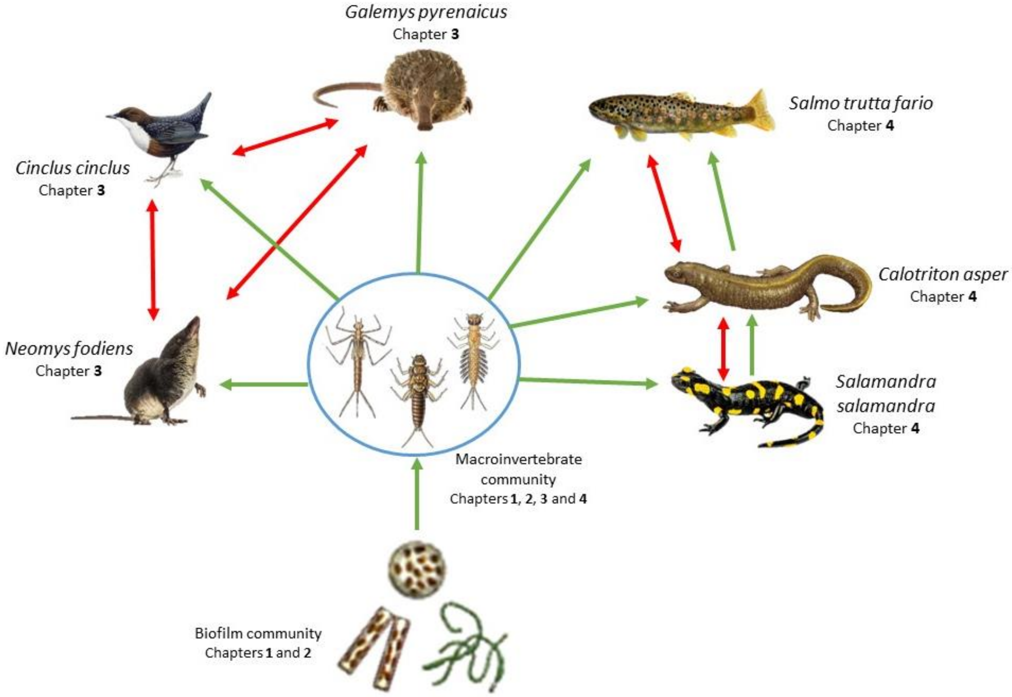

Figure 1. Summary of the relationships that have been analysed between biotic groups in each chapter of this thesis. Green unidirectional arrows indicate that a trophic group or species is preyed on by another. Red bidirectional arrows indicate competence between both groups.

2. Climate change impacts on the ecosystem structure and function of Pyrenean headwater streams

Flow regime is the key environmental driver of the ecosystem structure and function in streams (Winemiller et al., 2014) through the modulation of their water quality, energy sources, physical habitat and biotic interactions (Taylor et al., 2014). Intra-annual seasonal variations of the flow regime strongly influence Pyrenean headwater streams at different scales. In the Catllar stream, we observed a certain seasonal pattern of the water flow structuring the ecosystem. For instance, the low water flow during winter allows a stable and persistent riverbed that promotes high primary production levels, which is disrupted in spring and autumn by the high water flows that cause an excessive drag-disturbance and influence the physicochemical variables. During these high-flow months, the dominance of diatoms over macroalgae such as *Hydrurus foetidus,* and highly mobile macroinvertebrates is promoted, reducing the overall biomass of primary producers and macroinvertebrates (**Chapters 1** and **2**). This annual cycle is key for re-setting benthic communities to pioneer successional stages, which allows the maintenance of high biodiversity: if these disturbances, driven by the flow regime, occur on adequate frequencies that allow the redevelopment of the biotic communities, they will generally be composed of mixed pioneer and climax taxa (Biggs et al., 2005). The results of this thesis show these flow dynamics, as the analysed stream presents a marked seasonality that is reflected on the abiotic parameters as well as on the biotic communities. Despite this, differences in precipitation regimes evidence how these dynamics can be disrupted by a decrease in precipitations caused by climate change and its interaction with other stressors.

Water flow is the result of the interaction of rainfall, climate, land use and geomorphology (Ceola et al., 2013). It has been observed that the snow cover loss in the southern slope of the Pyrenees promoted by climate change is causing a significant flow reduction on headwater streams in this area (OPCC-CTP, 2018), seriously threatening their stability. These mountains are not only experiencing a decrease in precipitations and in the snow yield, but also an increase on the temperature of their waters and on the flood frequency (López-Moreno et al., 2008a, 2008b, 2009b; López-Moreno and Beniston, 2009a; Batalla et al., 2018). Even more, all the current studies on the future precipitation alterations on the Pyrenees agree about the future changes in the monthly precipitation and streamflow regimes (OPCC-CTP, 2018). The consequences of these effects on the ecosystem structure and function of a Pyrenean headwater stream have been analysed in **Chapters 1** and **2**. Lower water flows caused by a decrease in precipitations increased the concentration of nutrients and the water temperature. This had relevant implications on the metabolism of the ecosystem, leading to a chain of effects on its structure and function by promoting the autotrophy of the stream and biodiversity loss in a process similar to eutrophication (Dodds, 2007; Sabater and Tockner, 2009). This fact, alongside with the increase on the flood frequency, could alter the natural seasonal dynamics of these streams, reducing their high biodiversity levels, and causing disturbance events with such a frequency that would not allow the re-setting of the biotic communities of the stream. Even more, winter water flow of Pyrenean headwater streams is expected to increase, as warmer temperatures cause more liquid precipitation, reducing the snow yield and the spring and summer water flow levels (OPCC-CTP, 2018), which will furtherly alter these seasonal dynamics. Pyrenean headwater streams are strongly heterotrophic, and the trophic state of an ecosystem depends on multiple variables (Dodds, 2007), but the results of this thesis suggest that the current and future flow reduction, water warming and flood frequency increase caused by climate change could alter the seasonal dynamics of these ecosystems, promoting their autotrophy and decreasing their biodiversity. If winter water flow is increasing on future years (OPCC-CTP, 2018), the high productivity that a low flow and cold water promotes during winter will be impaired, altering the seasonal pattern of the stream.

These climate change impacts would have important implications for high trophic levels on headwater streams. On one hand, at a biogeographical scale, the distribution range of endemic species would be displaced to higher altitudes and latitudes accordingly with their thermal preferences (Daufresne et al., 2009; OPCC-CTP, 2018). This would imply the disappearance of cold-adapted stenotherm insectivorous predators on many Pyrenean headwater streams due to habitat loss, like the Pyrenean brook newt (Colomer et al., 2014), the river brown trout (Hornbach et al., 2016) and the Pyrenean desman (Aymerich and Gosálbez, 2018), causing trophic cascade effects on the biotic communities of the stream. On the other hand, at a local scale, predators would be indirectly affected by the impacts on the macroinvertebrate community caused by the environmental changes. Macroinvertebrates are a key energy source for vertebrate predators in these ecosystems (Allan, 1995), and this thesis highlighted how its availability influences the biotic relationships among those predators (**Chapter 3**). Macroinvertebrates are susceptible to climate change as a temperature increase can cause changes on the phenology of many species in different ways, leading to a desynchronization of key species interactions. Cold headwater streams are considered thermal refugia for cold-adapted stenotherm aquatic insects, causing the diversity of Ephemeroptera, Plecoptera and Trichoptera taxa to decrease on warmer habitats (Hornbach et al., 2016). In this regard, the macroinvertebrate biodiversity loss caused by climate change through water warming and eutrophication could limit the food resource availability for Pyrenean headwater top predators, favouring generalist species in detriment of more specialist ones (OPCC-CTP, 2018). This could cause interspecific competition and distribution displacements of these top predators to altitudes and/or latitudes more suitable for their ecological needs, especially for the Pyrenean desman (**Chapter 3**).

Climate change can also influence the physiology of many headwater species, affecting their population dynamics. The correlation between climate variability and demographic parameters of many vertebrate species has been vastly proven and it is known that it can influence their long-term survival (OPCC-CTP, 2018). Amphibians are especially vulnerable to these physiological changes due to their permeable skin and biphasic life cycle, making them sensitive to temperature and humidity changes. Even more, their sensitivity to chemical pollutants and their reproductive success has been proven to be affected by climate change (Araujo et al., 2011; Dastansara et al., 2017). Considering the current climate change perspectives, the increasingly mild winters on the Pyrenees can shorten the hibernation period of amphibians, preventing many individuals to enter on a complete hibernation state, consuming their reserves and reducing their body mass, which has a direct impact on their survival capability (Caruso et al., 2014). In **Chapter 4**, it was described that the upstream subpopulation of *C. asper* was smaller than the downstream one, which could be attributed to metal pollution. Climate change could, on one hand, increase the concentration of pollutants due to the expected reduction of stream flow dilution of metal pollution and, on the other hand, impair the hibernation period of this population. Water temperature is a key factor for this species, and the stream water temperature increase expected by climate change and water scarcity could cause a loss of up to 98% of the potential distribution area for C. asper due to thermic limitations (Colomer et al., 2014; Montori and Llorente, 2014; Araújo et al., 2011). Consequently, climate change is a serious threat to the conservation of *C. asper* in many headwater streams such as the Catllar stream, especially in the southern slope of the Pyrenees, and it could suffer a considerable reduction of its potential distribution (De Pous et al., 2016).

3. Damming impacts on the ecosystem structure and function of Pyrenean headwater streams

Damming is considered one of the main threats to worldwide freshwater biodiversity (Vörösmarty et al., 2010). The Catllar dam, despite being small, has proven to have a substantial impact on the ecosystem structure and function of the analysed headwater stream. This dam could be considered a component of a small hydropower plant (SHP), defined as a plant with a capacity of less than 50MW, with definitions depending on the country. This kind of structures have increased in number tremendously all around the world due to the current international efforts to achieve climate goals and reduce the quantity of large hydropower plants, which are highly destructive to the environment and human livelihoods (Lange et al., 2019). More than 82.000 SHPs have already been constructed worldwide, and at least 10.569 more are currently planned. Despite their abundance, SHPs constitute only a small proportion of the total hydropower energy production. For example, in the US they account for the 65% of the total number of hydropower facilities, but they contribute only a 3.5% to the total energy generation (Sharma et al., 2019). These SHPs are usually constructed without an assessment of their environmental and socio-economic impacts because, in many countries, this is only needed when the installation surpasses a certain energy capacity (Lange et al., 2019). This shows that SHPs have a relevant impact on the ecosystem structure and function of headwater streams, which must be assessed at different levels if we aim to conserve and manage these freshwater ecosystems.

Regardless of their size, dams inevitably cause local changes to the physical structure of streams (Alexandre and Almeida, 2009), as they strongly alter its hydrological regime and the water flow level and variability (Sabater and Tockner, 2009; Ceola et al., 2013; Taylor et al., 2014). This thesis showed that the flow disruption caused by damming can have direct as well as indirect impacts on all trophic levels of a headwater stream, and thus on its structure and function (**Chapter 2**). Although relevant, the flow restriction that damming caused on the Catllar stream had a weaker role on the structuring of the stream compared to the flow variability that seasonality implies. This might happen because the ecological effects of hydrological variability on the ecosystem structure of streams are nested hierarchically, and larger scales like seasonality constrain the influences of local scales, such as the influence of a SHP, at low levels of ecosystem organization (Biggs et al., 2005). Damming had multiple effects on the downstream characteristics, but the most remarkable ones were the increase of water temperature, algal biomass, and organic matter content on biofilms, as well as the biodiversity decrease (**Chapter 1**).

As it was mentioned on **Chapter 2**, biotic communities of headwater streams depend on allochthonous organic matter inputs during low productivity seasons such as autumn and summer, when the stream is mainly heterotrophic, but they depend on the biofilm and macroalgae production during winter and early spring, when the stream is mainly autotrophic. This pattern is key for the consumer-prey dynamics (Evangelista et al., 2014), and it was impacted by damming. The flow reduction caused by the dam increased the water temperature and the organic matter content of the biofilms, promoting their growth, regardless of the trophic state of the stream, causing a community shift (Sabater and Tockner, 2009; Ghedini et al., 2015). It increased the herbivorous biomass fraction of the macroinvertebrate community while reducing its biodiversity, having an impact on the whole trophic chain. Consequently, flow alterations caused by dams can impact not only the basal levels of biotic communities of headwater streams, but also higher trophic levels (Mor et al., 2017), which was observed in **Chapter 3** as the availability of macroinvertebrates was key in the prey electivity of insectivorous predators.

The impacts on basal resources and the ecological quality loss that damming implies inevitably affects the macroinvertebrate availability of the stream, which has the potential to promote individual trophic specializations, changing the foraging behaviour and habitat use of the predators (Evangelista et al., 2014). Consequently, the interspecific competition between insectivorous vertebrate predators on headwater streams would be exacerbated by the presence of a dam, causing the spatial segregation of the weakest competitor and local population extirpations and extinctions (Hardin, 1960). This effect would be intensified in the current climate change context in which the water availability is expected to decrease, aggravating the flow reduction and water warming in the downstream section of an impounded stream. In the Catllar stream, *G. pyrenaicus* would be severely affected by this situation due to the specialized diet of its individuals as a result of the competition (**Chapter 3**). In this regard, and considering that currently more than 115 SHPs, with their respective dams, are installed along the Pyrenees mountain range (Durban 2011), the conservation of the Pyrenean desman, and other threatened vertebrate species like the Pyrenean newt *C. asper*, should be especially considered on dam-impounded headwater streams.

4. Ecotoxicology of hydrologically altered Pyrenean headwater streams

Chemical pollutants have a strong influence on the structure and function of stream ecosystems, as their concentration is related to the water flow, the main driver of the structure of these ecosystems, especially at small scales such as in headwater streams (OPCC-CTP, 2018). Metal pollutants interact with the water flow and other environmental variables and stressors on complex situations, challenging the understanding of their specific impacts on the ecosystem structure and function of streams (Gessner and Tlili, 2016). In this regard, integrating ecological principles into ecotoxicological research is key in the assessment of those impacts, as its traditional focus on individuals is insufficient, and biomonitoring is a great tool for this kind of assessments. Many species can be used with this approach, and the effects of metal pollutants can be observed as bioaccumulation, morphological and behaviour changes, and population-level impacts, among others. This approach does not only reveal the subtle biological changes of organisms affected by pollution, but also the integrated effects of all pollutants and stressors in the biological community (Zhou et al., 2007).

The results of this thesis provide new ecotoxicological research of metal pollution effects on headwater streams. On one hand, at population-level by analysing population density and body size changes of an amphibian species in the presence of metal pollutants (**Chapter 4**). There is a current need of knowledge about the ecosystemic relevance of laboratory test on metal pollution (Clements et al., 2015; Gessner and Tlili, 2016), and in this chapter it was observed how previously known metal concentrations that have detrimental effects on salamandrids in a laboratory setting (Gardner et al., 2018) were likely to have a relevant ecological impact on a population of the newt *Calotriton asper*. On the other hand, at community-level, by means of changes in the structure (**Chapters 1** and **2**) and function (**Chapter 2**) of the ecosystem. Even more, those studies considered the metal effects under the additional stress of hydrological alteration and climate change, providing new insights on how metal pollution interacts with damming and precipitation reduction to impact the headwater streams.

Algae and biofilms are a great tool for metal pollution bioassessment. The fact that these pollutants can be accumulated in those organisms causes the biomagnification of metals along the food chain, threatening the structure and function of stream ecosystems (Zhou et al., 2007). In this thesis, data about this bioaccumulation was only available for biofilms (**Chapter 1**), but the bioaccumulation effects were reflected on higher trophic levels. Metal pollution in aquatic ecosystems is known to have a negative impact on the macroinvertebrate community (De Jonge et al., 2008) but, despite this, the current ability to link metal exposure with its effects on the ecosystem structure of streams is not satisfactory, as the metal sensitivity identified in laboratory tests does not predict the chronic effects of low doses in real ecosystems (Liess et al., 2017). Long-term analyses of metal exposures in headwater streams show unequivocal evidence of metal impacts on macroinvertebrate communities (Liess et al., 2017). Predator macroinvertebrates are more tolerant to metal pollution than herbivore groups (Liess et al., 2017), especially when arsenic is involved. This is caused by the different feeding characteristics of these groups (Chi et al., 2017), as scrappers, shredders and piercers bioaccumulate arsenic and other metals through the ingestion of polluted vegetal matter (Hepp et al., 2017), while predators are able to regulate their internal metal concentrations (Liess et al., 2017). Even arsenic levels of 40µg/L in the water can cause changes on the composition of macroinvertebrate assemblages (Hepp et al., 2017). In Liess et al. (2017), a decrease in herbivorous groups was reported with a water concentration of at least 5µg/L of Cu, while predators needed at least 10µg/L to experience a density decrease.

In the Catllar stream, metal concentrations upstream from the dam were higher (**Chapter 1**) and macroinvertebrate predators were more abundant than shredders, scrappers and piercers, while downstream these herbivorous groups dominated the community (**Chapter 2**). This situation could be caused by the combined action of metal pollution and damming. On one hand, the water diversion caused by the dam significantly reduced the metal concentrations downstream, which is beneficial for the herbivorous groups due to their sensitivity. Metal-induced stress increases their susceptibility to predation, which synergistically increases their individual sensitivity to pollutants, impairing even more their survival and reducing their biomass levels (Liess et al., 2017). On the other hand, the water flow reduction caused by the dam promoted the autotrophy of the downstream reach, promoting an algal and biofilm growth and improving its value as food resource through the organic matter accumulation, which contributed to increase the proportion of herbivores. This caused not only an impact on the macroinvertebrate community, but also on the function of the stream ecosystem as observed through the FFGs ratios (**Chapter 2**). Metal pollution situations that allow a higher predator biomass are expected to also impair other ecosystem functions such as leaf degradation processes (Liess et al., 2017). This was hinted on the Catllar stream by the CPOM to FPOM, and the suspended FPOM to deposited FPOM functional ratios being lower upstream from the dam, where the metal concentrations and predator biomass was higher. This community assemblage shift caused by the replacement of sensitive groups by tolerant ones is a common response in contaminated ecosystems and a reliable indicator of these impacts (Clements et al., 2015). These results provide new evidence for community-level responses to chronic exposures of low metal concentrations, showing its effects on the ecosystem structure and function of a headwater stream.

5. Conservation and management of Pyrenean headwater streams

Headwater streams are ecosystems with high levels of biodiversity due to their high physical and chemical heterogeneity, their frequent occurrence of near-natural conditions, and their high abundance in number. Because of this, the protection of these ecosystems is a concern in the scientific community, but there is still a lack of knowledge about these ecosystems, which is needed in order to understand their vulnerability to anthropogenic stressors and to propose adequate management actions (Biggs et al., 2016). The awareness of these streams is rising, but they still need more attention. An example of this situation is the Water Framework Directive in Europe, which is, in theory, designed to protect all European freshwater waterbodies, but many countries use size thresholds to define waterbodies that exclude headwater streams and do not take them in consideration for the management of whole river basins (EC, 2012). This is especially concerning because headwater streams are fundamental for the ecological integrity of the whole river network. On one hand, headwaters provide unique biota to the whole river basin because, despite each small stream having a low taxon richness, their collective network makes a large contribution to the regional biodiversity (Biggs et al., 2016). For example, up to a 29% of the macroinvertebrate biodiversity of a basin can be found in headwaters (Callanan et al., 2014). This makes the biodiversity of headwater streams a key element in the maintenance and restoration of downstream rivers to an acceptable conservation status, as the previously mentioned EU Water Framework Directive requires (Biggs et al., 2016). On the other hand, headwater streams provide a considerable volume of the water flow and a relevant quantity of nutrients to downstream rivers and lakes. In fact, up to a 90% of a river's flow can be derived from headwater streams (Saunders et al., 2009; OPCC-CTP, 2018).

The Pyrenees are a great example of these concerns about the management and conservation of headwater streams. The projected evolution of water demand on this mountain range during the current century estimates large uncertainties in water supply, which stresses the need for adaptation strategies (OPCC-CTP, 2018). The competition for water resources between human societies and stream ecosystems will be increased by the water scarcity that is being promoted by climate change and a growing human pressure. Aquatic ecosystems are impacted in a nonlinear way by the increase in water withdrawal, and a growing proportion of watercourses are being affected by extended low water flow periods (Sabater and Tockner, 2009; OPCC-CTP, 2018). As this thesis evidenced (**Chapters 1** and **2**), the decrease of water flow and the increase on its temperature due to damming and to a reduction in precipitations, impacts the ecosystem structure and function of a Pyrenean headwater stream. These factors promote the autotrophy of the stream and a biodiversity loss, which indicates the need of effective management actions in this changing context. The management of water resources and biodiversity in Pyrenean headwater streams is faced with a lack of knowledge on the impacts of anthropogenic stressors on their ecosystem structure and function (OPCC-CTP, 2018), but they also present further main challenges.

One of these main challenges is habitat fragmentation, which should be reduced as much as possible in order to ensure the ecological connectivity between Pyrenean streams and protected areas (OPCC-CTP, 2018). The presence of small dams that belong to SHPs (Small Hydropower Plants), like the one on this thesis, suppose one of the main causes of connectivity loss. Because the construction of these SHPs is being promoted worldwide (Sharma et al., 2019), a new management approach on these structures is needed. As proposed by Lange et al. (2019), SHPs should be subjected to the same environmental regulations as large hydropower plants as they are both associated with ecological threats and high socioeconomic costs, and their development needs to be guided by long-term planning policies and assessments at the basin scale. Even more, public and private subsidies to

SHPs should be revised considering their real ecologic and socioeconomic costs and benefits, as most of them are not economically viable without subsidies and their dismantling cost should be included in the planning. Dams of SHPs can be installed with fish passages and should allow a downstream release of an environmental flow (as discussed in **Chapter 1**) with the objective of reducing their ecological footprint. However, in general, these actions are insufficient or counterproductive. An example of this situation would be the *Calotriton asper* population of the Catllar stream (**Chapter 4**), which status would not be improved by these measures as they could not get through the fish passages and the environmental flow would allow an increase of the downstream concentration of metals.

A way to effectively nullify the impacts of SHPs could be the increase of the protected areas where the headwater streams are located, in order to avoid their construction. The presence of dams also impacts the upstream reach of its stream, and the legal protection of an area does not avoid the impacts of damming and other anthropogenic stressors. The upstream reach of the Catllar stream is under law protection, categorised as Natural Park and under the Natura 2000 conservation network, but the downstream reach. In **Chapters 1** and **2** the downstream effects of damming were analysed in biofilms and macroinvertebrates, but in **Chapter 4** we assessed the upstream impacts, as the *C. asper* population was not only affected by the lack of connectivity of the stream, but damming also interacted with the metal pollution that was originated from an abandoned mine in the protected area. The declaration of protected areas does not primarily focus on freshwater biodiversity conservation, they have a terrestrial focus. This causes existing protected areas to often fail when addressing key ecological processes such as the upstream-downstream impacts and the migration of freshwater-dependent species such as *C. asper* or *S. trutta fario*. These limitations highlight the urgent need of improvement on the performance of protected areas to address specific needs of headwater streams and their biodiversity (Hermoso et al., 2015), and also raise awareness about the need to protect the most representative areas of the Pyrenees in terms of biodiversity, especially headwater streams as they are particularly susceptible to climate change (OPCC-CPT, 2018).

Even though we should focus on the future and on what we can do to avoid further damage to these ecosystems, what can we do with the headwater streams that are already affected by dams and other anthropogenic impacts? Environmental restoration projects are a possible effective solution, and they are becoming a symbol of societies that want to use their natural resources in a sustainable way and to conserve their biodiversity. The problem is that in most of those regions, such as Western Europe, humans have impacted running waters for more than 4000 years, making it impossible to restore the ecosystem to its pristine state, especially considering the current and future human population densities. Considering this, with an anthropogenic perspective in mind, we should seek to optimise the resilience of these ecosystems with an acceptable risk for the human socioeconomic, political and cultural systems (Jungwirth et al., 2002). Considering that connectivity and water flow are the main factors that structure headwater streams, as assessed in this thesis, SHPs like the Catllar one should be one of the main threats to consider when addressing the conservation and restoration of these ecosystems. The impacts on biodiversity and human well-being derived from SHPs could be mitigated by stricter environmental policies, largescale planning and revised subsidy programs, and future SHPs should be only considered if they pass the same environmental controls as large hydropower dams (Lange et al., 2019).

Policy makers and legislators should recognise the importance that headwater streams have on the structure and function of all freshwater ecosystems, and on the maintenance of the services that they provide. This kind of streams are the commonplace of all freshwater habitats, but they have been historically overlooked by freshwater science until the last decades. The small catchment size of these streams makes them more vulnerable to stressors but, at the same time, more likely to escape them. They are easier to protect and to maintain in a near-natural condition than larger waterbodies. Consequently, their protection and management present a substantial and achievable opportunity to protect a key component of all freshwaters and, thus, policy makers, legislators, landowners and scientists should cooperate to facilitate this goal (Biggs et al., 2016).

A central goal of headwaters management should be to protect their biodiversity. The protection and restoration of a population from a single species or a group of species is a common action performed with this objective (Lake et al., 2007; Biggs et al., 2016). The use of these species as a flag for conservation of headwater streams could be useful for the protection of the habitat where they are found and, thus, the protection of the structure, function and services of the ecosystem. For Pyrenean headwater streams, the

Pyrenean desman (*Galemys pyrenaicus*) (**Chapter 3**) and the Pyrenean brook newt (*Calotriton asper*) (**Chapter 4**) could be great flag species, bearing this goal in mind. Conservation and management actions that consider the impacts of SHPs and metal pollution on these emblematic species could benefit many Pyrenean headwater streams and contribute to mitigate the current and future impacts that climate change will have on their ecosystem structure and function.

6. Research needs, future perspectives and contributions of this thesis

The international scientific community agrees on the fact that the combination of climate change with other anthropogenic stressors will be the main cause of biodiversity loss and species extinctions in the coming decades. In order to understand how headwater species will respond to these stressors, and how they will adapt to them, there is a need to increase the research effort and to promote more scientific networks to study these species to guarantee effective conservation strategies in the long term (OPCC-CTP, 2018). The results of this thesis highlight that climate change can have both synergistic and antagonistic effects with many current anthropogenic stressors that impact headwater streams, especially with damming and metal pollution as they are related to water flow. Considering that these interactions can cause multiple unexpected outcomes, more research is needed in order to understand how climate change can modulate the current and future effects of anthropogenic stressors on the ecosystem structure and function of headwater streams, while human societies adapt to the new climatic conditions.

A reduction in the precipitations had a relevant synergistic impact with damming on flow reduction (**Chapter 1**), which promoted the autotrophy of the Catllar stream and reduced its biodiversity (**Chapters 1** and **2**). A better understanding of the implications of this synergistic effect on the ecosystem structure and function of Pyrenean headwater streams would be useful in order to propose effective management actions, especially when considering additional local threats to each stream like metal pollution. The consequences of these interactive threats are especially relevant for key headwater species. For example, in the Catllar stream, *Hydrurus foetidus* was a macroalgae highly abundant on winter months, which populations will be especially impacted by climate change (**Chapter 1**). Being a psychrophile species, climate change and its interaction with other threats that promote warmer waters might have important consequences for this species on streams with similar characteristics. As *H. foetidus* supposes a basal resource for the macroinvertebrate community, alterations in the populations of this algae could diminish the prey availability for higher trophic levels such as the Pyrenean desman and the Pyrenean brook newt. **Chapter 3** highlighted the importance of prey availability for predators on this type of habitat and how this availability can modulate the trophic competition on higher trophic levels. However, more research is needed on how this macroinvertebrate availability will be affected by interactive anthropogenic stressors, in order to assess the conservation needs of species with current declining populations like the Pyrenean desman. The Pyrenean brook newt is also severely threatened by these stressors, and **Chapter 4** showed how metal pollution has an important role on the conservation of this species, as it can reduce the population density and body size of this species. Because metal pollution is also modulated by damming and climate change (**Chapter 1**), more field studies of their impacts on the survival and physiological state of headwater amphibians are needed in this changing environment. Moreover, laboratory experiments aimed to understand which metals and metalloids, and in which concentrations, can have impacts on the amphibians of these ecosystems could help establish pollution thresholds that can be used for their management and conservation. This is especially relevant for the endangered *Calotriton asper*, as there is a huge knowledge gap about how metal pollution affects this species.

Not only research, but also new management actions will be needed in the future for the proper conservation of headwater streams in a climate change context. The areas and species most vulnerable to climate change and other anthropogenic stressors should be identified to define priority conservation areas and restoration opportunities, increasing the monitoring plans on them and promoting forms of land use that are compatible with their conservation. These adaptation policies should be harmonised within competent private and public bodies that manage the land with the objective of protecting, improving and restoring biodiversity while maximising positive synergies between them. Moreover, considering the example of the Catllar stream, where only the upstream part of the dam was under a legal protection figure, the administrative boundaries of current and future protected areas should be adjusted to the biology and distribution of key headwater species while considering the changing climate (OPCC-CTP, 2018).

Integrative approaches, including trophic interactions, are necessary to understand the effects of flow regime on stream communities (Ceola et al., 2013). This thesis presented an integrative approach to damming and metal pollution impacts in a climate change context on the ecosystem structure and function of headwater streams, using a Pyrenean stream as a case study. It provided a global vision of these impacts while considering different biotic and abiotic indicators, assessing how they influence each other. The conservation status of many key species needs to consider their current and potential vulnerability to climate change (OPCC-CTP, 2018), and these results provided relevant information about how environmental and anthropogenic stressors affect emblematic species like *Galemys pyrenaicus* and *Calotriton asper*.

General conclusions

1. The ecosystem structure and function of Pyrenean headwater streams are strongly dependent on the characteristics of their water flow regime, which is driven by a strong seasonal pattern. This seasonal pattern can be impaired by anthropogenic stressors, such as damming and climate change. The structural and functional parameters of headwater streams do not present a homogeneous response in front of multiple anthropogenic stressors because their interactions can exacerbate or mitigate their individual effects.

2. Both damming and a precipitation decrease promoted by climate change can reduce the water flow of Pyrenean headwater streams and increase its temperature. This situation promotes the autotrophy of the stream, reducing its biodiversity and altering the direction of the trophic chain, hindering the seasonal dynamics of the stream. This situation can be worsened by metal and metalloid pollution, as the water flow reduction increases their concentration in water, having an impact on the community assemblage of biofilms and macroinvertebrates.

3. The analysis of the functional feeding groups of the macroinvertebrate community allows the assessment of not only the ecosystem structure of headwater streams, but also its functioning and how these ecosystems respond to multiple anthropogenic stressors. As this simple approach is effective in the assessment of the relationship between biodiversity and ecosystem functioning, its use is encouraged for the management and conservation of headwater streams.

4. The impacts of damming and a precipitation decrease on the macroinvertebrate community of headwater streams can affect its availability as food resource for vertebrate predators such as the Pyrenean desman *Galemys pyrenaicus*, exacerbating the interspecific trophic competition with species that use similar resources (such as *Neomys fodiens* and *Cinclus cinclus*) and displacing its populations.

5. In a situation of interspecific trophic competition, the Pyrenean desman *Galemys pyrenaicus* specializes its diet on energetically profitable and highly available rheophile prey, while avoiding terrestrial individuals. Abundant fast-flowing riffle sections in mountain streams are needed in order to provide quality habitat and enough prey availability for the Pyrenean desman, as well as for its physiological adaptations to be effective. In this regard, the flow reduction and connectivity loss that

damming causes is a serious threat for the conservation of this species, which needs of a higher awareness.

6. Diet analyses are effective in the assessment of the food requirements of vertebrate species such as the Pyrenean desman. However, it is essential to collect the diet samples as fresh as possible in order to avoid environmental contamination of groups that feed on those samples. Consequently, these analyses need to consider the ecology of the prey taxa in order to extract reliable conclusions.

7. Damming implies a water diversion that causes differences in the water characteristics and quality at each side of the dam. If the upstream water is polluted with metals and metalloids, the diversion can benefit the downstream biodiversity by promoting the presence of sensitive species such as the Pyrenean brook newt (*Calotriton asper*) and the macroinvertebrate families Ephemeroptera, Plecoptera and Trichoptera. Consequently, damming and metal pollution can have an antagonistic effect on the ecosystem structure of Pyrenean headwater streams.

8. Metal and metalloid pollution (e.g., arsenic and chromium) can decrease the population density and the individual size of the Pyrenean brook newt *C. asper*. Finding a *C. asper* population with these characteristics, in streams with suitable habitat but located in mining areas, could be an indicator for metal and metalloid pollution.

9. Metal and metalloid pollution reduces the abundance of sensitive macroinvertebrate taxa, affecting the ecosystem functioning through the alteration of the trophic chain and the reduction on the efficiency of leaf degradation processes.

10. Headwater streams are ecosystems highly vulnerable to anthropogenic stressors. These ecosystems are fundamental for the health of whole river basins, but they are not usually considered for management and conservation actions. More research and awareness on these ecosystems are needed in order to effectively manage their biodiversity and functioning under the current global change context. Policy makers, legislators, landowners and scientists should cooperate to facilitate this goal.

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