INFLUENCE OF FLOW ALTERATION AND METAL POLLUTION ON STREAM BIOTA: FROM SPECIES TO COMMUNITY-LEVEL RESPONSES

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DOCTORAL THESIS - Influence of flow alteration and metal pollution on stream biota: from species to community-level responses

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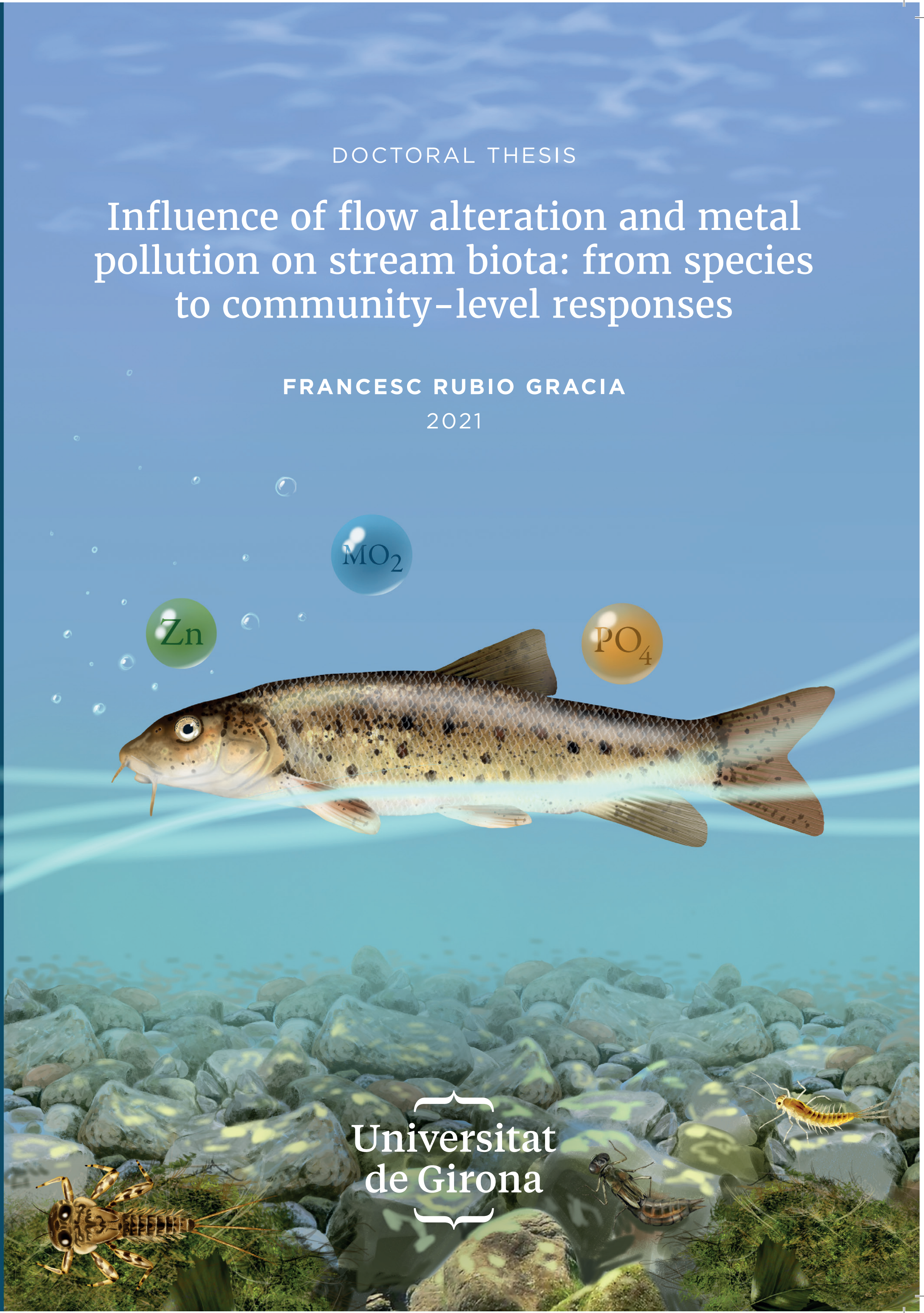
FRANCESC RUBIO GRACIA

2021



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Doctoral Thesis

Influence of flow alteration and metal pollution on stream biota: from species to community-level responses

Francesc Rubio Gracia

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Doctoral Program in Water Science and Technology

Thesis supervisor	**Co-supervisor**
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Thesis submitted in fulfillment of the requirements for the
doctoral degree at the University of Girona.

Hereby, Dr. Anna Vila Gispert of the University of Girona and Dr. Elena Guasch Padró of the Blanes Centre for Advanced Studies,

CERTIFY:

That the thesis entitled "*Influence of flow alteration and metal pollution on stream biota: from species to community-level responses"*, presented by Francesc Rubio Gracia to obtain a doctoral degree, has been completed under our supervision and meets the requirements to opt for the International Doctor mention.

In witness whereof and for such purposes as may arise, the following certification is signed:

Dr. Anna Vila Gispert Dr. Elena Guasch Padró

Als meus pares,

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Nou de juliol de dos mil vint-i-u. El camí ha estat llarg, molt llarg de fet si giro la vista enrere. Diuen que les coses arriben, passen i canvien per algun motiu. La tesi la vaig començar el 2015 poc després d'acabar el màster a Barcelona. Però si he acabat fent el doctorat a Girona és, en part, gràcies al fet que vaig decidir-me (o em van convèncer) per fer la llicenciatura de Ciències Ambientals a Girona a l'any 2008. Girona, m'has enamorat.

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DERIVED SCIENTIFIC PUBLICATIONS

Rubio-Gracia F., García-Berthou E., Guasch H., Zamora L. and Vila-Gispert A. (2020). Size-related effects and the influence of metabolic traits and morphology on swimming performance in fish. *Current Zoology, 66*, 493–503.

Rubio-Gracia F., Garcia-Berthou E., Latorre D., Moreno-Amich R., Srean P., Luo Y. and Vila-Gispert A. (2020). Differences in swimming performance and energetic costs between an endangered native toothcarp (*Aphanius iberus*) and an invasive mosquitofish (*Gambusia holbrooki*). *Ecology of Freshwater Fish, 29*, 230–240.

Rubio-Gracia F., Almeida D., Bonet B., Casals F., Espinosa C., Flecker A. S., García-Berthou E., Martí E., Tuulaikhuu B-A., Vila-Gispert A., Zamora L. and Guasch H. (2017). Combined effects of hydrologic alteration and cyprinid fish in mediating biogeochemical processes in a Mediterranean stream. *Science of the Total Environment, 601–602*, 1217–1225.

Rubio-Gracia F., Argudo A., Zamora L., Clements W. H., Vila-Gispert A., Frederic C. and Guasch H. Indirect food-web interactions are mediated by metal pollution and an ecosystem engineer, *Barbus meridionalis* (Cyprinidae), in a Mediterranean stream. *Manuscript submitted to Aquatic Sciences.*

LIST OF ACRONYMS

AAS	Absolute aerobic scope
AFDW	Ash free dry weight
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
APHA	American Public Health Association
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
Chl-*a*	Chlorophyll-*a*
Cond	Conductivity
COT	Cost of transport
DM	Dry mass
EC_{50}	Effect concentration for 50% of individuals
EPA	U.S. Environmental Protection Agency
EPT	Ephemeroptera, Plecoptera and Trichoptera
FFG	Functional feeding groups
M	Fish mass
MMR	Maximum metabolic rate
NH_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate
OCH	Odonata, Coleoptera, Hemiptera
PERMANOVA	Permutational multivariate analysis of variance
PO_4^{3-}	Phosphate
SMR	Standard metabolic rate
TL	Total length
U_{crit}	Critical swimming speed
U_{opt}	Optimal swimming speed

LIST OF FIGURES

GENERAL INTRODUCTION

GENERAL METHODS

RESULTS

Chapter I

Chapter II

Chapter III

Chapter IV

GENERAL DISCUSSION

SUPPLEMENTARY MATERIAL

Chapter I

Chapter II

LIST OF TABLES

GENERAL METHODS

RESULTS

Chapter I

Chapter II

Chapter III

Chapter IV

GENERAL DISCUSSION

SUPPLEMENTARY MATERIAL

Chapter II

Chapter IV

CONTENTS

CHAPTER I

CHAPTER II

CHAPTER III

CHAPTER IV

SUMMARY

Community structure and ecosystem functions are determined by physical and chemical conditions, food resources and biotic interactions. Mediterranean-climate streams are intrinsically variable in streamflow, with marked flow reduction in summer and floods in autumn and spring according to regional precipitation patterns. Riverine species are adapted to cope with natural and predictable disturbances, and their responses are in a large part determined by their physiological and behavioral adaptations. As for fish, swimming performance can determine their ability to use habitats differing in water velocity, to obtain food and avoid predation, etc. Today, the intense use of water by human demands causes longer low-flow periods in streams, thereby producing a certain degree of water stress. In addition, nutrient enrichment and water pollution are also common anthropogenic stressors that threaten ecological integrity of freshwater ecosystems. Metal pollution is of particular concern due to its high potential toxicity, perdurability and bioaccumulation through the food chain. At the bottom of the food chain, periphyton communities serve as food and energy sources for many organisms (e.g. macroinvertebrate grazers), so any stressor affecting periphyton is likely to indirectly affect organisms of a higher trophic level ("bottom-up control"). On the contrary, periphytic algal biomass can be affected by shifts in grazing pressure, e.g. resulting from predator-induced changes in grazer density ("top-down control"). Periphyton communities are also involved in important ecosystem functions (e.g. nutrient cycling), and are important bio-indicators for habitat degradation. Field mesocosm experiments, from which we can modify the density of certain consumers (e.g. fishes), have become an invaluable tool in ecotoxicology and ecology for increasing mechanistic understanding of stressor effects.

This thesis aimed to assess the responses of stream biota to flow alterations, in terms of water velocity increments and reductions of discharge, and to metal pollution. The investigations performed encompassed (i) respirometry studies using swim-tunnel

respirometers to evaluate swimming capabilities, as indicative of water velocity tolerance, and physiological responses (energetic costs of swimming) of several freshwater fishes, as well as the drivers (morphological features) of variation within and among species; and (ii) field observational and mesocosm studies were carried out to evaluate the role of the threatened fish *B. meridionalis* as modulator of the response of stream ecosystem structure and function to anthropogenic stressors, such as hydrologic alterations (e.g. resulting from the water diversion) and metal pollution.

Our results showed that there exist a high intra-and interspecific variation in absolute critical swimming speed and metabolic traits in fish. Generally, swimming performance and aerobic metabolism scaled positively with body size. The study that compared the invasive mosquitofish (*Gambusia holbrooki*) with the native Spanish toothcarp (*Aphanius iberus*) revealed that mosquitofish can swim faster (i.e. higher critical swimming speed) and more efficiently than Spanish toothcarp, as it showed lower cost of transport at a comparable workload. Both sexes of mosquitofish showed a better hydrodynamic body shape, i.e. streamlined body and thiner caudal peduncle, both of which were positively correlated with swimming performance. The second study revealed that, after accounting for body size effects, critical swimming speed was independently affected by total length or maximal metabolic rate within certain fish species. At the interspecific level, variation in critical swimming speed was partly explained by the variation in the interrelated traits of maximal metabolic rate, and fineness and muscle ratios, when accounting for up to eight freshwater fish species.

Field observational and mesocosm studies showed that the top-down control by grazers was largely responsible for the variation in structural responses of periphyton, such as biomass and nutrient content, and in functional responses, such as nutrient uptake rates. Generally, when grazer densities were higher the periphyton biomass (ash-free dry mass or chlorophyll-*a*) was lower, whereas the nitrogen content of periphyton increased (i.e. lower C:N). The higher

phosphate uptake capacity by periphyton was likely due to a more rejuvenated and active periphyton community. In addition, the variation in macroinvertebrate and fish densities were directly related to the impacts of natural (floods) and anthropogenic stressors. In metal-polluted reaches, we found that direct negative effects of metals on grazers led to indirect positive effects on periphyton biomass.

Our studies also provided evidence about the important functional role of the Mediterranean barbel (*Barbus meridionalis*) in streams because shifts in their densities can lead to large ecosystem changes. The absence of this predatory fish triggered trophic-cascade responses on periphyton biomass in low impacted streams, and led to modify phosphate uptake rates by periphyton through biotic interactions. On the contrary, this benthic fish markedly reduced periphyton biomass via bioturbation impacts, and the greatest consumption effects on macroinvertebrates were found in larvae of Chironomidae.

Finally, it was discussed (i) the importance of fish swimming capabilities, metabolism and morphological traits in influencing patterns of species distributions in Mediterranean streams with hydrologic alterations; and (ii) the context dependency of stream community responses to anthropogenic stressors. A number of management actions and future research prospects in relation to our findings were also raised throughout this thesis.

RESUM

L’estructura comunitària i les funcions dels ecosistemes estan determinades per les condicions físiques i químiques, els recursos alimentaris i les interaccions biòtiques. Els rius de clima mediterrani són intrínsecament variables en el cabal, amb una reducció marcada del cabal a l’estiu i inundacions a la tardor i a la primavera segons els patrons de precipitació regionals. Les espècies fluvials estan adaptades per fer front a pertorbacions naturals i previsibles, i les seves respostes estan determinades en gran part per les seves adaptacions fisiològiques i conductuals. Pel que fa als peixos, el rendiment de la natació pot determinar la seva capacitat per utilitzar hàbitats amb diferent velocitat del corrent, per obtenir menjar i evitar depredacions, etc. Avui en dia, l’ús intensiu de l’aigua per satisfer les necessitats humanes provoca períodes prolongats de baix cabal als rius, produint així un cert grau d’estrès hídric. A més, l’enriquiment de nutrients i la contaminació de l’aigua també són estressors antròpics habituals que amenacen la integritat ecològica dels ecosistemes d’aigua dolça. La contaminació per metalls és especialment preocupant per la seva alta toxicitat potencial, perdurabilitat i bioacumulació a través de la cadena alimentària. A la part inferior de la cadena alimentària, les comunitats de perifiton serveixen com a font d’aliment i energia per a molts organismes (p. ex. macroinvertebrats herbívors), de manera que qualsevol factor estressant que afecti el perifiton pot afectar indirectament els organismes d’un nivell tròfic superior (“control de baix a dalt”). Per contra, la biomassa algal perifítica es pot veure afectada per canvis en la pressió d’herbívoria, p. ex. a partir de canvis en la densitat d’herbívors induïts pels depredadors ("control de dalt a baix"). Les comunitats de perifitons també participen en funcions importants de l’ecosistema (p. ex. el reciclatge de nutrients) i són bioindicadors importants de la degradació de l’hàbitat. Els experiments de mesocosmos de camp, a partir dels quals podem modificar la densitat de certs consumidors (p. ex. peixos), s’han convertit en una eina

invaluable en ecotoxicologia i ecologia per augmentar la comprensió mecanicista dels efectes dels estressors.

Aquesta tesi tenia com a objectiu avaluar les respostes de la biota fluvial a les alteracions del flux, en termes d’increments de la velocitat de l’aigua i reduccions de cabal, i a la contaminació per metalls. Les investigacions realitzades van incloure (i) estudis de respirometria mitjançant túnels de natació per avaluar les capacitats de natació, indicatives de la tolerància a la velocitat de l’aigua, i les respostes fisiològiques (despeses energètiques de la natació) de diversos peixos d’aigua dolça, així com els factors (característiques morfològiques) responsables de la variació a dins i entre les espècies; i (ii) estudis d'observació de camp i mesocosmos per avaluar el paper del peix amenaçat *B. meridionalis* com a modulador de la resposta de l'estructura i funció dels ecosistemes aquàtics als estressors antròpics, com ara les alteracions hidrològiques (p. ex. resultants de la desviació de l'aigua) i la contaminació per metalls.

Els nostres resultats van demostrar que existeix una elevada variació intra- i inter-específica en la velocitat crítica de natació absoluta i en els trets metabòlics en els peixos. En general, el rendiment de la natació i el metabolisme aeròbic van augmentar positivament amb la mida corporal. L'estudi que va comparar el peix mosquit invasor (*Gambusia holbrooki*) amb el fartet (*Aphanius iberus*) va revelar que el peix mosquit pot nedar més ràpidament (és a dir, una velocitat crítica de natació més alta) i amb més eficiència que el fartet, ja que mostrava un cost de transport inferior a una càrrega de treball comparable. Els dos sexes del peix mosquit van mostrar una millor forma corporal hidrodinàmica, és a dir, un cos fusiforme i un peduncle caudal més prim, i tots dos trets es van correlacionar positivament amb el rendiment de la natació. El segon estudi va revelar que, després de tenir en compte els efectes de la mida corporal, la velocitat crítica de natació es va veure afectada independentment per la longitud total o la taxa metabòlica màxima en determinades espècies de peixos. A nivell interespecífic, la variació de la velocitat crítica de natació s'explica en part per la variació dels trets interrelacionats de la taxa metabòlica màxima i de les relacions de finor i músculs, en comptabilitzar fins a vuit espècies de peixos d'aigua dolça.

Els estudis d'observació i mesocosmos de camp van demostrar que el control de dalt a baix per part dels herbívors era en gran part responsable de la variació de les respostes estructurals del perifiton, com ara el contingut de biomassa i nutrients, i de les respostes funcionals, com ara les taxes d'absorció de nutrients. En general, quan la densitat d'herbívors era més elevada, la biomassa del perifiton (massa seca lliure de cendres o clorofil·la) era més baixa, mentre que el contingut de nitrogen del perifiton augmentava (és a dir, un C: N més baix). La major capacitat de captació de fosfat pel perifiton es deu probablement a una comunitat de perifiton més rejovenida i activa. A més, la variació de la densitat de macroinvertebrats i peixos es relacionava directament amb els impactes dels estressors naturals (inundacions) i antròpics. A les zones contaminades per metalls, vam trobar que els efectes negatius directes dels metalls sobre els herbívors van provocar efectes positius indirectes sobre la biomassa del perifiton.

Els nostres estudis també van proporcionar evidències sobre l'important paper funcional del barb mediterrani (*Barbus meridionalis*) en els rius atès que canvis en les seves densitats poden provocar canvis importants en l'ecosistema. L'absència d'aquest peix depredador va produir una cascada tròfica sobre la biomassa del perifiton en els trams de riu poc alterats, i va provocar la modificació de les taxes d'absorció de fosfats del perifiton mitjançant interaccions biòtiques. Per contra, aquest peix bentònic va reduir notablement la biomassa del perifiton mitjançant impactes de bioturbació, i els efectes de major consum sobre els macroinvertebrats es van trobar a les larves de Chironomidae.

Finalment, es va discutir (i) la importància de la capacitat de natació dels peixos, el metabolisme i els trets morfològics per influir en els patrons de distribució d'espècies en rius mediterranis amb alteracions hidrològiques; i (ii) la dependència del context de les respostes

de les comunitats fluvials als estressors antròpics. Al llarg d'aquesta tesi també s’han plantejat diverses accions de gestió i perspectives de recerca futures en relació amb les nostres troballes.

RESUMEN

La estructura comunitaria y las funciones de los ecosistemas están determinadas por las condiciones físicas y químicas, los recursos alimenticios y las interacciones bióticas. Los ríos de clima mediterráneo son intrínsecamente variables en caudal, con una reducción marcada del caudal en verano e inundaciones en otoño y en primavera según los patrones de precipitación regionales. Las especies fluviales están adaptadas para hacer frente a perturbaciones naturales y previsibles, y sus respuestas están determinadas en gran parte por sus adaptaciones fisiológicas y conductuales. En cuanto a los peces, el rendimiento de natación puede determinar su capacidad para utilizar hábitats con diferente velocidad de corriente, para obtener comida y evitar depredaciones, etc. Hoy en día, el uso intensivo del agua para satisfacer las necesidades humanas provoca períodos prolongados de bajo caudal en los arroyos, produciendo así un cierto grado de estrés hídrico. Además, el enriquecimiento de nutrientes y la contaminación del agua también son estresores antrópicos habituales que amenazan la integridad ecológica de los ecosistemas de agua dulce. La contaminación por metales es especialmente preocupante por su alta toxicidad potencial, perdurabilidad y bioacumulación a través de la cadena alimentaria. En la parte inferior de la cadena alimentaria, las comunidades de perifiton sirven como fuente de alimento y energía para muchos organismos (p. ej. macroinvertebrados herbívoros), de forma que cualquier factor estresante que afecte el perifiton puede afectar indirectamente a los organismos de un nivel trófico superior ("control de abajo hacia arriba"). Por el contrario, la biomasa algal perifítica puede verse afectada por cambios en la presión de herbivoría, p. ej. a partir de cambios en la densidad de herbívoros inducidos por los depredadores ("control de arriba a abajo"). Las comunidades del perifiton también participan en funciones importantes del ecosistema (p. ej. el reciclaje de nutrientes) y son bioindicadores importantes de la degradación del hábitat. Los experimentos de mesocosmos de campo, a partir de los cuales podemos modificar la densidad de ciertos consumidores (p. ej. peces), se han convertido en

una herramienta invaluable en ecotoxicología y ecología para aumentar la comprensión mecanicista de los efectos de los estresores.

Esta tesis tuvo como objetivo evaluar las respuestas de la biota fluvial a las alteraciones del flujo, en términos de incrementos de la velocidad del agua y reducciones de caudal, y a la contaminación por metales. Las investigaciones realizadas incluyeron (i) estudios de respirometría mediante túneles de natación para evaluar las capacidades de natación, indicativas de la tolerancia a la velocidad del agua, y las respuestas fisiológicas (gastos energéticos de la natación) de varios peces de agua dulce, así como los factores (características morfológicas) responsables de la variación dentro y entre las especies; y (ii) estudios de observación de campo y mesocosmos para evaluar el papel del pez amenazado *B. meridionalis* como modulador de la respuesta de la estructura y función de los ecosistemas acuáticos a los estresores antrópicos, como las alteraciones hidrológicas (p. ej. resultantes de la desviación del agua) y la contaminación por metales.

Nuestros resultados demostraron que existe una alta variación intra- e inter-específica en la velocidad crítica de natación absoluta y en los rasgos metabólicos en los peces. En general, el rendimiento de natación y el metabolismo aeróbico aumentaron positivamente con el tamaño del cuerpo. El estudio que comparó el pez mosquito invasor (*Gambusia holbrooki*) con el fartet (*Aphanius iberus*) reveló que el pez mosquito puede nadar más rápidamente (es decir, una velocidad crítica de natación más alta) y con más eficiencia que el fartet, ya que mostraba un coste de transporte inferior a una carga de trabajo comparable. Ambos sexos del pez mosquito mostraron una mejor forma corporal hidrodinámica, es decir, un cuerpo fusiforme y un pedúnculo caudal más delgado, y ambos rasgos se correlacionaron positivamente con el rendimiento de natación. El segundo estudio reveló que, después de tener en cuenta los efectos del tamaño corporal, la velocidad crítica de natación se vio afectada independientemente por la longitud total o la tasa metabólica máxima en determinadas especies de peces. A nivel interespecífico, la variación de la velocidad crítica de natación se explica en parte por la variación de los rasgos interrelacionados de la tasa metabólica máxima y de las relaciones de finura y músculos, al contabilizar hasta ocho especies de peces de agua dulce.

Los estudios de observación y mesocosmos de campo demostraron que el control de arriba a abajo por parte de los herbívoros era en gran parte responsable de la variación de las respuestas estructurales del perifiton, como el contenido de biomasa y nutrientes, y de las respuestas funcionales, como las tasas de absorción de nutrientes. En general, cuando la densidad de herbívoros era más alta, la biomasa del perifiton (masa seca libre de cenizas o clorofila) era más baja, mientras que el contenido de nitrógeno del perifiton aumentaba (es decir, un C: N más bajo). La mayor capacidad de captación de fosfato por el perifiton se debe probablemente a una comunidad de perifiton más rejuvenecida y activa. Además, la variación de la densidad de macroinvertebrados y peces se relacionaba directamente con los impactos de los estresores naturales (inundaciones) y antrópicos. En las zonas contaminadas por metales, encontramos que los efectos negativos directos de los metales sobre los herbívoros condujeron a efectos positivos indirectos sobre la biomasa del perifiton.

Nuestros estudios también proporcionaron evidencias sobre el importante papel funcional del barbo mediterráneo (*Barbus meridionalis*) en los ríos ya que cambios en sus densidades pueden provocar cambios importantes en el ecosistema. La ausencia de este pez depredador produjo una cascada trófica sobre la biomasa del perifiton en los tramos de río poco alterados, y provocó la modificación de las tasas de absorción de fosfatos del perifiton mediante interacciones bióticas. Por el contrario, este pez bentónico redujo notablemente la biomasa del perifiton mediante impactos de bioturbación, y los efectos de mayor consumo sobre los macroinvertebrados se encontraron en las larvas de Chironomidae.

Finalmente, se discutió (i) la importancia de la capacidad de natación de los peces, el metabolismo y los rasgos morfológicos para influir en los patrones de distribución de especies

en ríos mediterráneos con alteraciones hidrológicas; y (ii) la dependencia del contexto de las respuestas de las comunidades fluviales a los estresores antrópicos. A lo largo de esta tesis también se han planteado diversas acciones de gestión y perspectivas de investigación futuras en relación con nuestros hallazgos.

1. GENERAL INTRODUCTION

1.1 The ecology of streams

1.1.1 The importance of flow

Rivers and streams are flowing water bodies that occur as hierarchical, interconnected networks draining surface and subsurface water from watersheds to downstream waters (e.g. large rivers, lakes, oceans). There is no real distinction between streams and rivers, except that the former refers to smaller systems. Rivers receive and transport water in three dimensional pathways (Figure 1): (i) longitudinal (from source to mouth); (ii) lateral (across the channel and floodplain); and (iii) vertical (surface waters, groundwater and their interactions). These three dimensional pathways vary over time representing the fourth dimension of a river system (Ward, 1989). The observed discharge at distinct locations within the drainage basin is determined based on meteorological and biogeophysical factors (Zeiringer et al. 2018).

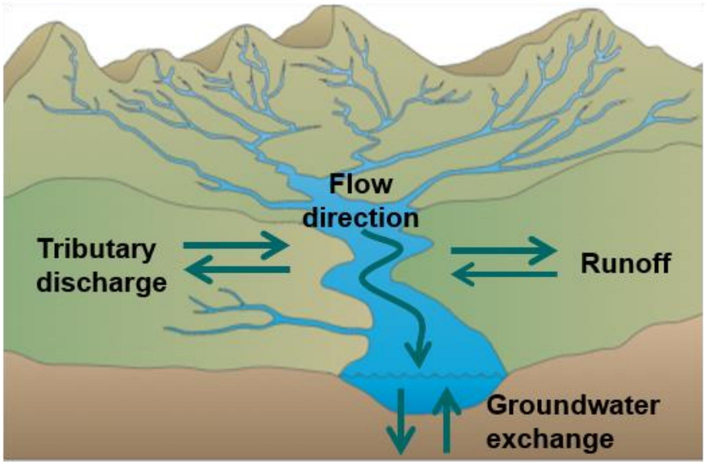
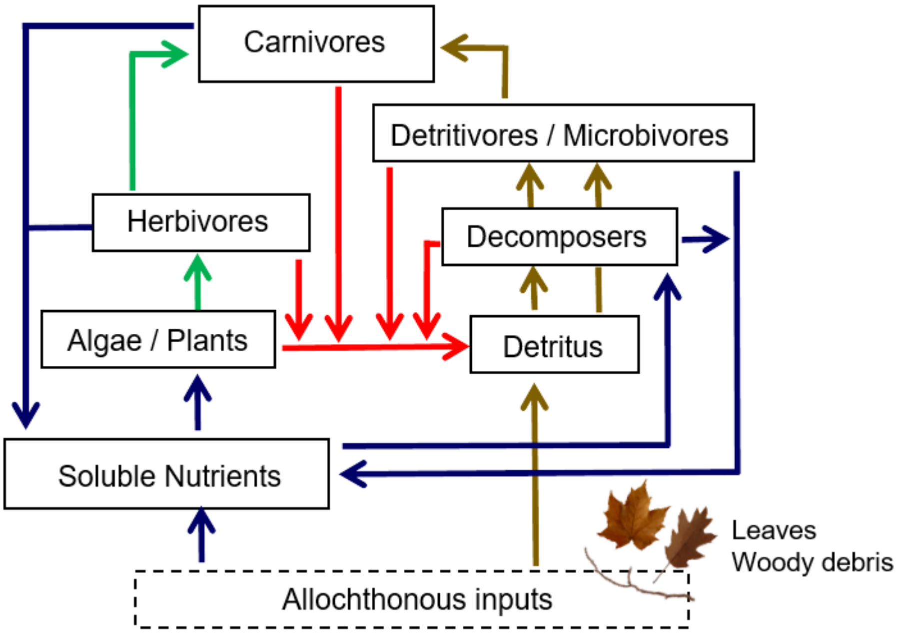


FIGURE 1 Water inputs and outputs through the three dimensional pathways of a river: longitudinal, lateral and vertical. Watershed vector was taken from Ian Image Library with copyrights permission

Streamflow is a fundamental driver of fluvial ecosystems, shaping the habitat structure and biodiversity (Allan and Castillo, 2007). Indeed, streamflow is strongly correlated with many critical physicochemical characteristics of streams (e.g. temperature and suspended solids), channel geomorphology, and habitat heterogeneity, which in turn limits the distribution and abundance of riverine species, and regulates the ecological integrity of fluvial systems (Poff et al. 1997). Moreover, the natural variability of flow regimes in terms of magnitude, frequency, duration, timing, and rate of change (Poff et al. 1997) can affect population and community dynamics, biotic interactions, and short and long-term biological processes (Gasith and Resh, 1999; Naiman et al. 2008; Palmer and Ruhi, 2019). The responses of stream biota to natural and predictable disturbances (e.g. floods and droughts) are determined by their physiological and behavioral adaptations (Resh et al. 1988; reviewed in Lytle and Poff, 2004). At the reach scale, flow velocity is an important physical habitat feature, and jointly with depth, substrate composition and instream vegetation and debris determine habitat suitability for riverine species (Fischer and Paukert, 2008; Boavida et al. 2011; Nakagawa, 2014). As for fish, habitat preferences were previously related to body size, sex, life stages, habitat guilds and swimming capabilities (Hockley et al. 2014; Del Signore et al. 2016). We will further address this issue in section 1.2 (see page 22).

1.1.2 The flow of matter and energy

The movement of water through the different above-described pathways facilitates the entry of physical, chemical, and biological inputs from the surrounding environment into the stream channel (Lamberti et al. 2010). In fact, river ecologists have long recognized that the biological processes at the reach scale are influenced by the surrounding landscape (Allan et al. 1997; Allan, 2004; Larsen et al. 2016). Carbon (C), which is considered the currency of energy transfer across trophic levels (Dodds, 2002), enters rivers through the conversion of carbon dioxide (CO_2) into organic compounds in the process of photosynthesis, and through the import and subsequent processing of terrestrial organic matter [leaves and woody debris, dissolved organic matter (DOM)] from the drainage basin, which entails respiratory processes (C remineralization). Therefore, both primary production by autotrophs (algae or plants) and/or organic matter decomposition by heterotrophs (e.g. decomposers) constitute the main pathways through which energy and matter are assimilated and transformed by stream communities (Figure 2).



F I G U R E 2 A simplified representation of the aquatic food web featuring producers, consumers, and decomposers, and the transfer of energy. The green arrows represent the flow of matter through the grazer pathway that originates from primary producers (plants or algae). The brown arrows represent the detritus pathway that originates from external detritus imports (e.g. leaves and woody debris) and internal detritus cycling. The red arrows depict the autochthonous flow of matter to the detritus pool that results from death of all living organisms and from unassimilated prey. The blue arrows refer to the mineralization and immobilization of soluble nutrients. Adapted from Moore et al. (2004)

There is a general agreement in stream ecology that the detrital or detritus-based pathways constitute much of the energy that supports fluvial food webs (Moore et al. 2004), since most primary production is not consumed by herbivores (grazers), but rather is returned to the environment as detritus (Odum and Biever, 1984). However, the autotroph-based food web may represent the main energy pathway in some fluvial ecosystems (Minshall, 1978).

Moreover, both pathways are interlinked and can affect each other (Zou et al. 2016). For instance, predators of detritivores can increase nutrient mineralization, and indirectly enhance primary production (Stone and Weisburd, 1992). The relative importance of the two pathways in transferring energy between trophic levels can also vary in space and time due to variation in subsidies and abiotic conditions, in agreement with the river continuum concept (Vannote et al. 1980).

1.1.3 Stream biota: environmental controls and nutrient cycling

Periphyton is a complex community of autotrophs (diatoms, green algae, and cyanobacteria) and heterotrophs (bacteria, fungi, and protozoa), which are embedded in an extracellular polymeric matrix adhered on stream rocks and cobbles (Romaní et al. 2012). Although literature shows some ambiguity regarding the use of "periphyton" and "biofilm" as terms to either refer to autotrophs or heterotrophs, or both; in this thesis periphyton refers to both. When referring to autotrophs the term "primary producers" is also used. Given the strategic position at the bottom of the food chain, periphyton communities serve as food and energy sources for many organisms of higher trophic levels (e.g. invertebrates). Therefore, potential shifts in abundance of periphyton or in the quality of carbon and the nutrient (nitrogen and phosphorus) content of it, can affect consumer growth rates (called bottom-up control) (Stelzer and Lamberti, 2002). Abiotic factors affecting periphyton biomass either negatively or positively are summarized in Figure 3. Biotic factors (e.g. grazers) may also regulate biomass accrual (called top-down control) (Figure 3), and stimulate periphyton productivity (Feminella and Hawkins, 1995). Because of the complex of interactions between biotic and abiotic factors, untangling top-down and bottom-up controls in an ecosystem has long aroused the interest of ecologists (Hunter and Price, 1992; Power, 1992; Taylor et al. 2015). Today, there is evidence that both type of controls can act separately or synergistically. For instance, Hillebrand (2005) found that light favors algal growth if nutrients are not limiting, but increased algal biomass can be easily offset by increased grazer densities.

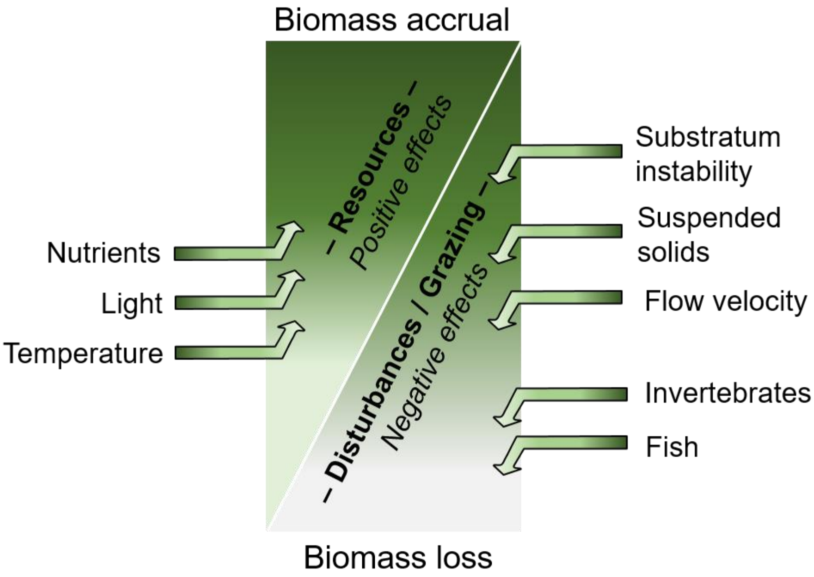


FIGURE 3 Abiotic and biotic factors influencing periphyton biomass accrual in river ecosystems. Nutrient enrichment and increased light intensity favours algal growth (Guasch and Sabater, 1994; Guasch et al. 1995). Increased water temperature, typically in summer, increase metabolic rates (Guasch and Sabater, 1994). In contrast, fine sediment reduces adnate and filamentous algae abundances (Cattaneo et al. 1997); increased suspended solids cause turbidity and reduce light availability (Arenas-Sánchez et al. 2016), and increased flow velocity cause drag forces and abrasion (Ponsatí et al. 2016). Herbivores such as grazing invertebrates and some fish species reduce periphyton biomass by consumption (Murdock et al. 2011)

Macroinvertebrates have significant roles in periphyton- and detritus based food webs (Anderson and Sedell, 1979). Functional feeding groups (FFG) classification provides a general picture of the macroinvertebrate community structure (Tachet et al. 2010): grazers (also termed scrapers), eating periphytic algae and associated material from cobbles; collectors, eating fine organic matter (FPOM), transported by flow (collector-filterers) or deposited (collector-gatherers, also termed deposit-feeders); shredders, eating and fragmenting coarse organic matter (CPOM) and consequently eating microbes associated with decomposition; and predators, eating on other consumers. However, some aquatic invertebrates are opportunistic feeders and thus cannot be rigidly placed in a single functional group. In addition to the bottom-up control, macroinvertebrate densities can also be controlled by top-down (via consumption by invertebrate and fish) forces (Flecker and Townsed, 1994). We will address the ecological role of fish in freshwater ecosystems in section 1.3 (see page 25).

Nutrient cycling is the flux of nutrients within and between the various biotic and abiotic components of streams. This thesis deals with the cycling of dissolved inorganic nitrogen [DIN species: ammonium ($NH4^+$) and nitrate (NO_3^-)] and phosphorus (as orthophosphate, PO_4^{3-}) in which periphyton is involved. Nitrogen and/or phosphorus (P) usually limit primary producers in aquatic ecosystems (Guasch et al. 1995; Elser et al. 2007). DIN species and P are incorporated into organic form by assimilatory uptake (Proia et al. 2017), move through the food web by consumption, and are subsequently mineralized to inorganic forms by excretion and the decomposition of organic matter (Figure 4) (Vanni, 2002). Moreover, atmospheric nitrogen gas (N_2) can be fixed by cyanobacteria, and subsequently converted into $NH4^+$. In addition, two energy-yielding reactions carried out by some bacteria transform N between various inorganic oxidation states (dissimilatory uptake) (Figure 4); that is, nitrification (the transformation of NH_4^+ to NO_3^-), and denitrification (the transformation of NO_3^- to N-gas). Moreover, even though not addressed explicitly in this thesis, it is worth noting that dissimilatory NO_3^- reduction to NH_4^+ (DNRA) can occur under sub-oxic to anoxic conditions (Ribot et al. 2017). As illustrated in Figure 4, several environmental drivers can influence nutrient uptake rates, such as nutrient concentrations or community composition. In addition, both N and P can be removed from the stream water by abiotic processes such as sorption-desorption reactions and co-precipitation (Doods, 2003). In short, changes in nutrient cycling can indicate changes in ecosystem structure and functioning, and therefore the response of periphyton uptake to environmental stress conditions makes it a potentially interesting functional indicator (Proia et al. 2017).

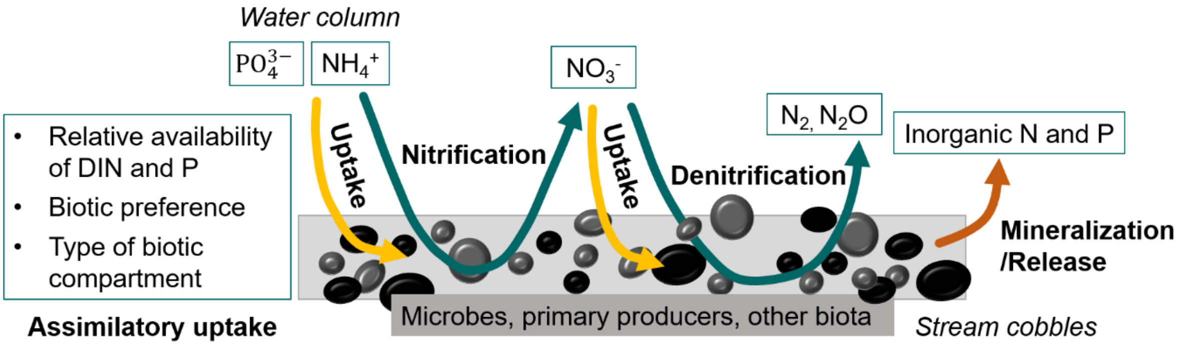


FIGURE 4 Main assimilatory and dissimilatory uptake pathways of dissolved nitrogen and phosphorous in streams. Assimilatory uptake is mainly driven by the relative availability of each dissolved inorganic N and P, the type of biotic compartment (e.g. biofilm, bryophytes, and vascular plants) and its preference for NH_4^+ over NO_3^- and affinity for PO_4^{3-} (Proia et al. 2017). For its part, dissimilatory uptake pathways of NO_3^- (i.e. denitrification) and NH_4^+ (i.e. nitrification) occur under contrasted redox conditions. Mineralization is the conversion of organic N and P to inorganic forms and then released back into water. Adapted from Ribot et al. (2017)

1.1.4 Mediterranean streams: characteristics and anthropogenic stressors

Mediterranean streams have flow regimes that reflect the precipitation patterns of the Mediterranean climate (Bonada and Resh, 2013). Seasonal rainstorms occur mainly during the autumn and spring and they usually cause floods. Summer drought can substantially reduce streamflow, and even some stream reaches may dry up completely. Although the timing of drying and flooding is very predictable, the intensity and frequency of these events are not (Gasith and Resh, 1999). As a result, aquatic species have evolved different, often opposing adaptive mechanisms to undergo seasonal shifts in physical habitat (e.g. increased water velocity and discharge during the wet season) and connectivity (e.g. pool isolation during the dry season). Therefore, community composition is expected to vary in response to these natural extreme events (Bogan et al. 2017, and therein references). In summer, lentic colonists and lotic refugees are dominant taxa in pools. On the contrary, flow resumption during the rewetting phase allows rapid recolonization by resistant and resilient lotic species (Bonada et al. 2007; Bonada and Resh, 2013).

Importantly, Mediterranean rivers that are large enough or streams that are supplied by ground water can maintain perennial flows in summer. However, some running waters are shifting from permanent to temporary because of unsustainable use of water by human demands (e.g. water diversion for irrigation; Larned et al. 2010) and the effects of climate change (Filipe et al. 2013), thereby compromising the dynamic equilibrium of streams and their services (Acuña et al. 2017).

In addition to hydrological alterations, including both flow modification and over-exploitation, other common stressors in Mediterranean streams are: (i) water pollution, coming from domestic and industrial point sources (e.g. high nutrient loads because of agricultural runoffs); (ii) habitat degradation (e.g. excavation of river sand); and (iii) species invasion (Dudgeon et al. 2006). Given the context of global change, the co-occurrence of multiple stressors is becoming more frequent (Ormerod et al. 2010), and thus the number of works interested in studying multiple stressor effects have been increasingly growing. Recent studies showed that stressors rarely act in isolation and can interact in many ways (Sabater et al. 2016; Schinegger et al. 2016). Some other studies, quantifying single and shared effects of multiple stressors, have revealed that direct effects of hydrological alterations or indirect effects mediated by hydrology (e.g. physical habitat structure or land use) were the main drivers of ecosystem structure and functioning, for both diatoms and macroinvertebrates (Mathers et al. 2019; Meißner et al. 2019; De Castro-Català et al. 2020). In contrast, other authors concluded that pollution was the most important stressor for periphyton P uptake (Pereda et al. 2020) and algal biomass (Ponsatí et al. 2016). Collectively, this apparent contradiction reflects the complexity of biotic responses to multiple stressor effects (Segner et al. 2014), as well as the significance of environmental context-dependency (Clements et al. 2016).

It has been well established that metal toxicity on stream biota can be aggravated under low flow conditions due to a lower dilution capacity of the system (Petrovick et al. 2011). Sabater et al. (2002a) experimentally tested how different flow velocities and exposure to Cu^{2+} (15 µg L^{-1}) would affect periphyton responses. They found that conjoint effects of low flows and metal exposure negatively affected chlorophyll-*a* concentration and photosynthetic activity. Moreover, several studies reported shifts in dominance of tolerant over sensitive taxa of periphyton (Medley and Clements, 1998) and macroinvertebrates (Clements and Rees, 1997; Sabater et al. 2016; Kalogianni et al. 2017).

1.2 Linking water flow and fish physiology

1.2.1 Swimming performance and energy metabolism

Fish is one of the most diverse taxonomic groups, accounting for heterogeneous ranges of size and body form, as a result of a long evolutionary history (Moyle and Cech, 1988). More than 15,000 fish species have been estimated only for fresh waters (Tedesco et al. 2017), thus representing an important component of global biodiversity (Reid et al. 2013). In shallow, aquatic environments, water flow structures fish species assemblages and influences patterns of diversification (Poff and Allan, 1995; Olden and Kennan, 2010) according to the ability of organisms to move in or withstand these physical forces (Patrick and Yuan, 2017). Both body and fin shape can affect the ability of fish to persist in high water flow environments (Binning and Roche, 2015), and are also strongly related to swimming capabilities (Webb, 1984; Blake, 2004; Langerhans and Reznick, 2010). Swimming performance is assumed to be a character determining survival (Plaut, 2001) because swimming is the only alternative for most aquatic prey to avoid the attack from their predators. Moreover, swimming capacity is directly related to food capture, habitat selection, migration and reproduction, and it is thus of high ecological importance (Videler, 1993; Webb, 1994).

The study of swimming performance of fishes has a relatively long history (see reviews by: Beamish, 1978; Hammer, 1995; Kolok, 1999; Plaut, 2001). Swimming performance of fish can be classified into three categories: sustained, prolonged and burst swimming (Beamish, 1978). From a mechanical perspective, burst swimming involves unsteady movements, whereas sustained and prolonged swimming involves steady movements (Webb, 1984). Fish tend to optimize either unsteady or steady swimming depending on fish biomechanics and morphological traits (Figure 5). Since Brett's work (Brett, 1964), many authors have opted for determining critical swimming speed (U_{crit}), which refers to the highest swim speed that a fish can sustain using (primarily) aerobic metabolism, as a measurement of prolonged swimming performance (Beamish, 1978). Prolonged performances are those speeds that fish can maintain for 20 seconds to 200 minutes and ends in fatigue (Beamish, 1978). Typically, for the measurement of swimming performance is necessary the use of a water tunnel or flume in which fish are forced to swim against water current (Farrell, 2008). In addition, two important metabolic traits are estimated during the U_{crit} protocol, from which the respiratory capacity of a fish can be described: the standard metabolic rate (SMR) refers to the energy that an animal must spend on the maintenance of tissues and homoeostatic mechanisms needed to sustain life (Fry, 1971). After satisfying the maintenance energy requirements, the excess of energy can be allocated to other functions, but always below the upper limit set by the maximum metabolic rate (MMR), which represents the maximal rate at which oxygen can be supplied to tissues and adenosine triphosphate (ATP) can be produced (Fry, 1971). The difference between SMR and MMR is absolute aerobic scope (AAS), which represents the animal's capacity to support energetically expensive processes aerobically (Clark et al. 2003). Given that most processes occurring in an animal require ATP and that the most efficient ATP-producing pathway requires oxygen, AAS is regarded as an indicator of whole-animal performance that links directly to the capacity for activity, growth and reproduction, and thereby, ultimately fitness (Fry, 1971).

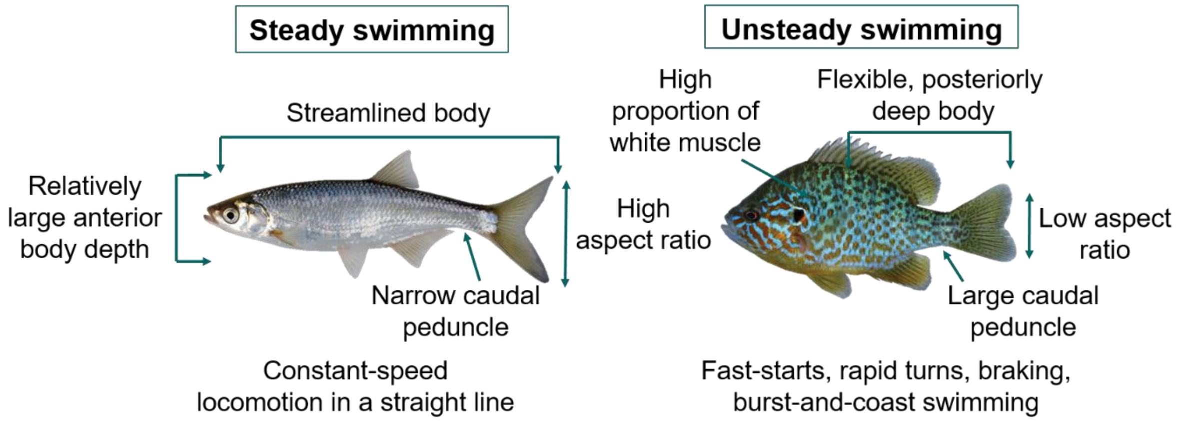


FIGURE 5 Fish biomechanics and morphological traits that enhance steady and unsteady swimming in fish. Steady swimming describes constant speed cruising, which is important for acquiring resources; whereas unsteady swimming describes rapid bursts and turns, which is important for escaping predators and for capturing preys. Fish images courtesy of Enric Aparicio

Knowledge of the swimming performance and energy metabolism of fish species can be useful for the management of water bodies, e.g. for the design of fishway passages or in the development of barriers to exclude invasive species (Peake et al. 2017). In addition, the studies focused on the swimming performance and aerobic scope of species are increasing in importance to address questions relating to fish ecology and the effects of climate change (Clark et al. 2013; Costa et al. 2017). Today, much of the available information about swimming performance and metabolic traits is from commercial and recreational target species or long-distance migratory species, such as salmonids (e.g. Farrell, 2008). However, this knowledge is still limited for many other species (e.g. Tudorache et al. 2008). This is the case, for example, of many Mediterranean fish (Alexandre et al. 2014; 2016) and rare or local endemic species with poor conservation status (Cano et al. 2020a). Therefore, this thesis is intended to fill this knowledge gap by determining swimming performance and energy metabolism of several freshwater fish species inhabiting the Iberian Peninsula, of which some are native and other are invasive. Although not conclusive, it is believed that native fish species are likely to be more reophilic and therefore better swimmers than invasive ones, which likely has more preference for calm, stagnant waters (Clavero and Hermoso, 2011). Providing information on physiological traits of both native and invasive species may help to understand the mechanisms that underlie species interactions and distributions.

1.3 Reciprocal interactions between fish and the structure and functioning of stream communities

1.3.1 Fish as ecosystem engineers

Stream dynamics are controlled by a combination of abiotic and biotic factors, some of which have been already mentioned: flow regime, downstream flows of energy and nutrients, reciprocal subsides of matter and organisms between riparian and stream habitats, and the biotic control (e.g. grazing effects). However, there is still a crucial biotic factor that largely influences stream processes and dynamics; the so-called "ecosystem engineering" (Jones et al. 1997). Jones et al. (1997) defined ecosystem engineers as the organisms that directly or indirectly control the availability of resources to other organisms through the physical modification, maintenance, or creation of habitats. All animals indeed can engineer their environments to some degree. But the ecosystem engineers are the ones that play a crucial role in maintaining and affecting other organisms in the ecosystem. Identifying which organisms have larger effects on habitats, and determining when and where these ecosystem processes occur are still issues to be sorted out in stream ecology fields. The challenge is that the impacts of stream biota on ecosystems are not ubiquitous; rather, they are frequently context-dependent (Gido et al. 2010; Vanni, 2010). In an attempt to address these challenges, important theoretical studies have developed conceptual frameworks to guide further studies focused on ecosystem engineers (Crain and Bertnress, 2006; Moore, 2006).

Moore (2016) proposed that the total impact of ecosystem engineers will be a function of behaviour, body size, and population density. Considering that fishes are often the largest aquatic animals in streams and relatively abundant, it is likely that their impacts on a per capita basis will be larger than those caused by smaller aquatic organisms. Moreover, numerous species of freshwater fishes are known to generate physical perturbation of benthic habitats (i.e. bioturbation) through three types of activities: nest digging (Moore et al. 2004), foraging (Flecker, 1996) and movements (Winemiller and Jepsen, 2004). Either of these fish activities may result in increased sediment–water exchanges, which has important effects on physico-chemistry and water column processes. Power (1990) demonstrated that armored catfish (Siluriformes: Loricariidae) reduced sediment accrual, which decreased the shading of benthic periphyton and led to increased primary productivity. Likewise, bioturbation can be a substantial source of disturbance to benthic invertebrates (Flecker and Taylor, 2004). Beyond consumer–resource interactions and bioturbation, fishes also directly or indirectly mediate biogeochemical fluxes through top-down control or the excretion of dissimilated nutrients (Vanni, 2002), thereby influencing the structure of primary producers and ecosystem processes.

Regarding the environmental context dependency, several studies demonstrated that both physical disturbances (e.g. floods) and ecosystem subsidies are major drivers of ecosystem engineering by fish (Moore, 2006). Fish effects are expected to be more important in intermediate to low flows conditions, since high flows can overwhelm or mask the top-down control of fish on lower trophic levels, nutrient cycling and sediment accrual (Vanni, 2010). Moreover, the influence of fish on nutrient cycling is expected to be more important under conditions of nutrient limitation (Spooner et al. 2013), especially after a disturbance, when periphyton biomass accumulates and dissolved nutrients are depleted (Vanni, 2010).

Ultimately, because subsidies of matter and energy control the body size and density of ecosystem engineers, a decrease in subsidy availability may lead to reduce the impacts of fishes on ecosystem (Moore, 2006).

1.3.2 Experimental approaches: fish caging

Traditionally, monitoring programs have been focused on chemical water quality and metrics based on community structure and diversity, partly because the Water Framework Directive (WFD) and other similar policy frameworks are still largely based on the use of abiotic or taxonomic variables as indicators of human impacts on ecosystem health (Feld et al. 2009). While it is true that community composition reflects the relative degree of habitat degradation (Cairns and Pratt, 1993), its unique understanding does not necessarily capture dynamics of ecosystem nutrient and energy cycling and transformation, or biological productivity. Therefore, a more holistic approach is needed to accurately predict human impacts on streams, and to assist in identifying which impacts are having the strongest effects on ecological integrity (Truchy et al. 2015). Apart from that, previous studies demonstrated the power of integrating descriptive and experimental approaches for establishing a mechanistic understanding of community responses to contaminant effects (Culp et al. 1999; Clements et al. 2002; Guasch et al. 2012). The reason is that biomonitoring studies have reliance on statistical inference (e.g. nonindependence of treatments, lack of appropriate replications) and are unable to identify underlying mechanisms responsible for changes in aquatic communities (e.g. indirect effects of stressors), thus hampering the establishment of cause-and-effect relationships between stressors and ecological responses. One of the best ways to show cause-and-effect relationships among organisms is to use cages or mesocosms in streams. This experimental approach provides a link between field surveys and highly controlled laboratory experiments (Kennely, 1991; Schmitz, 2008; Winemiller et al. 2014). However, it should be noted that mesocosm studies also have some limitations, such as small spatiotemporal scale, experimental artefacts, or difficult applicability to real scenarios. Three main designs can be used with stream fishes: (i) the exclusion of fishes; (ii) the inclusion of fishes; and (ii) the modification of abundances of fish. Therefore, on the one hand, it is possible to detect and quantify the ecological role of fish in an ecosystem by manipulating their densities (Rodríguez-Lozano et al. 2015; 2016a), and on the other hand, to detect and quantify structural and functional responses of stream communities to environmental stressors effects (Townsed et al. 2008). From a managerial perspective, the findings provide valuable insights for possible mitigation actions (Birk, 2019).

This thesis has focused on understanding the roles that biotic (predator top-down control) and abiotic drivers play in influencing ecosystem functions and/or structure in streams. Hydrologic alterations (flow modification, intermittency) were always present at the study streams, but additional stressors such as high nutrient concentrations or metal pollution also occurred. Specifically, we investigated the impacts of the predatory fish, *Barbus meridionalis* (A. Risso, 1827), on lower trophic levels (periphyton and macroinvertebrates) in different environments using cages in streams, and tested whether the effects related to the presence or absence of fish are mediated by the anthropogenic stressors. Consider, for example, what happens in a situation where hydrological alteration and metal pollution co-occur. Trophic interactions might be or not be altered by multiple stressor effects, and therefore many different ecological outcomes may occur depending on the response of biological communities to environmental stressors. In general, biological communities tend to reach an “equilibrium” in benign environments, whereas stressful environments can alter that equilibrium by modifying interaction strengths (Figure 6).

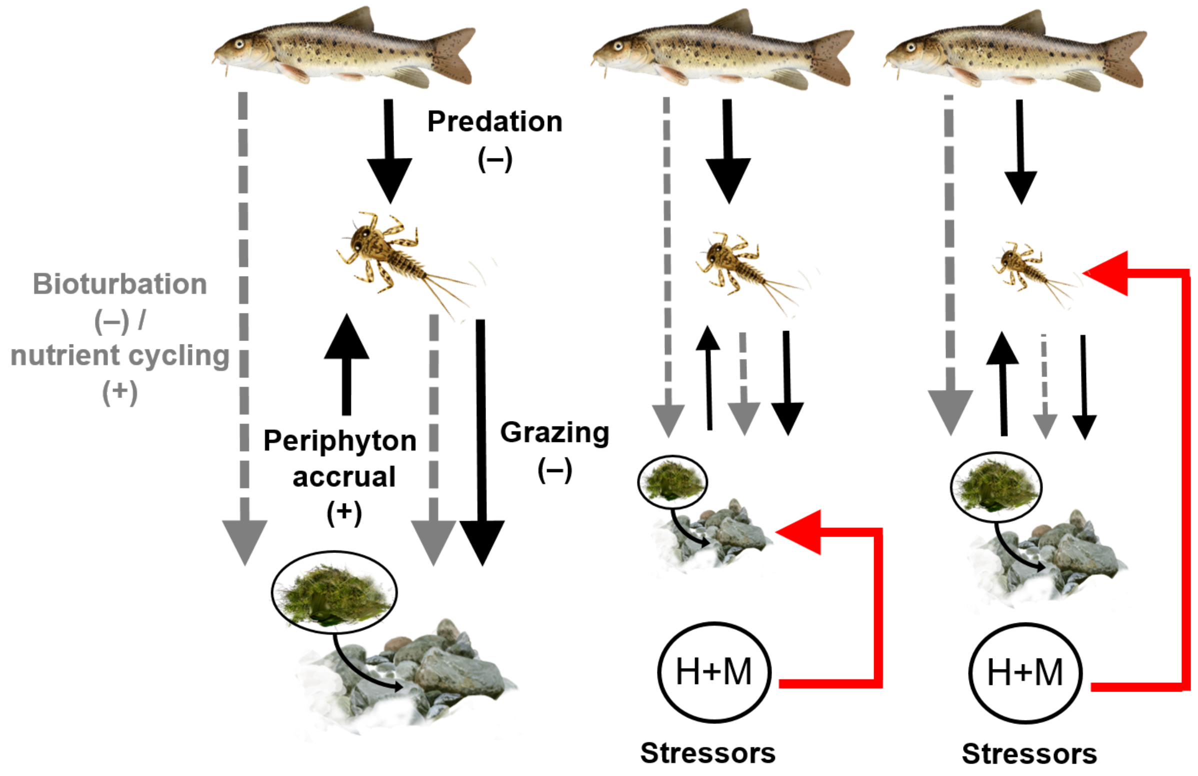


FIGURE 6 Conceptualized figure of trophic interactions based on the periphyton-grazer interactions, when (i) there are no stressors or their effects are not significant (left part); or when (ii) combined effects of hydrological alteration (H) and metal pollution (M) affect different trophic levels (middle and right parts). The thickness of the arrow is proportional to interaction strength, whereas the size of each biological community indicates the potential ecological outcome. Darker solid arrows indicate direct effects (top-down and bottom-up controls); grey dashed arrows indicate indirect effects of biological activity; and red arrows indicate multiple stressor effects. Text in bold describes the type of interaction (e.g. predation), and (+/–) the sign of the effect on the receiving functional group. Note that non-trophic interactions (competition or facilitation) among species in the same trophic level or trait-mediated trophic cascade can also occur, thereby modifying the expected ecological outcome

2. OBJECTIVES

The main aim of this thesis was to assess the responses of stream biota to flow alterations, in terms of water velocity increments and reductions of discharge, and to metal pollution. Specifically, the investigations performed encompassed, on the one hand, respirometry studies using swim-tunel respirometers (Chapters I & II) to evaluate swimming capabilities as indicative of water velocity tolerance, and physiological responses (energetic costs of swimming) of several freshwater fishes, as well as the drivers (morphological features) of variation within and among species. On the other hand, field observational and mesocosm studies (Chapters III & IV) were carried out to evaluate the role of the threatened fish *B. meridionalis* as modulator of the response of stream ecosystem structure and function to anthropogenic stressors, such as hydrologic alterations resulting from the water diversion (Chapter III) and metal pollution (Chapter IV). More in detail, the present dissertation is splitted into four chapters with their own specific objectives:

Chapter I. "Differences in swimming performance and energetic costs between an endangered native toothcarp (*Aphanius iberus*) and an invasive mosquitofish (*Gambusia holbrooki*)". This study aimed at determining swimming capabilities and metabolic traits in *A. iberus* for the first time, and comparing the swimming performance and efficiency of this native fish with that of the invasive *G. holbrooki*. Morphological descriptors of swimming performance for both species were also taken into account. It was initially expected that *G. holbrooki* performed best in terms of swimming performance and efficiency given the differences in habitat use and migration rates between these two species.

Chapter II. "Size-related effects and the influence of metabolic traits and morphology on swimming performance in fish". Following Chapter I, we aimed at comparing swimming performance and metabolic traits of eight freshwater fish species differing in body size and morphological traits. Specifically, we investigated to what extent intra- and interspecific variation in swimming performance and metabolism is explained by differences in body size, and morphological and physiological traits. It was expected that rheophilic species, such as cyprinids, performed best in terms of swimming performance and efficiency than species with a slow-flow lifestyle.

Chapter III. "Combined effects of hydrologic alteration and cyprinid fish in mediating biogeochemical processes in a Mediterranean stream". The objective of this study was to compare the structural and functional response of two stream reaches differing in terms of hydrologic alteration, to additional alterations associated with the absence or increased density of fish (*Barbus meridionalis*). Namely, we estimated biomass, elemental content and nutrient uptake (ammonia and phosphate) as response variables for periphyton, and biomass and density as response variables for macroinvertebrates. It was hypothesized that the effects of long period of dryness caused by hydrological alterations would persist after rewetting, thereby influencing the response of benthic communities to the presence or absence of *B. meridionalis*.

Chapter IV. "Indirect food-web interactions mediated by metal pollution and an ecosystem engineer, *Barbus meridionalis* (Cyprinidae), in a Mediterranean stream". This study aimed at evaluating the responses of periphyton and macroinvertebrates to the presence and absence of a predatory fish (*Barbus meridionalis*) along a metal pollution gradient. Specifically, we investigated whether trophic relationships interact with environmental stress, and whether the ecological role of top predators in streams is context dependent. We hypothesized that (i) effects of metal pollution would negatively impact stream benthic communities; (ii) the top predator absence (*B. meridionalis*) would promote community-level cascades in benign or low altered environments (Rodríguez-Lozano et al. 2016a), whereas environmental stress can attenuate biotic interactions; and (iii) the direct negative effects of metal exposure on a specific trophic level may trigger indirect positive effects on other trophic levels.

3. GENERAL METHODS

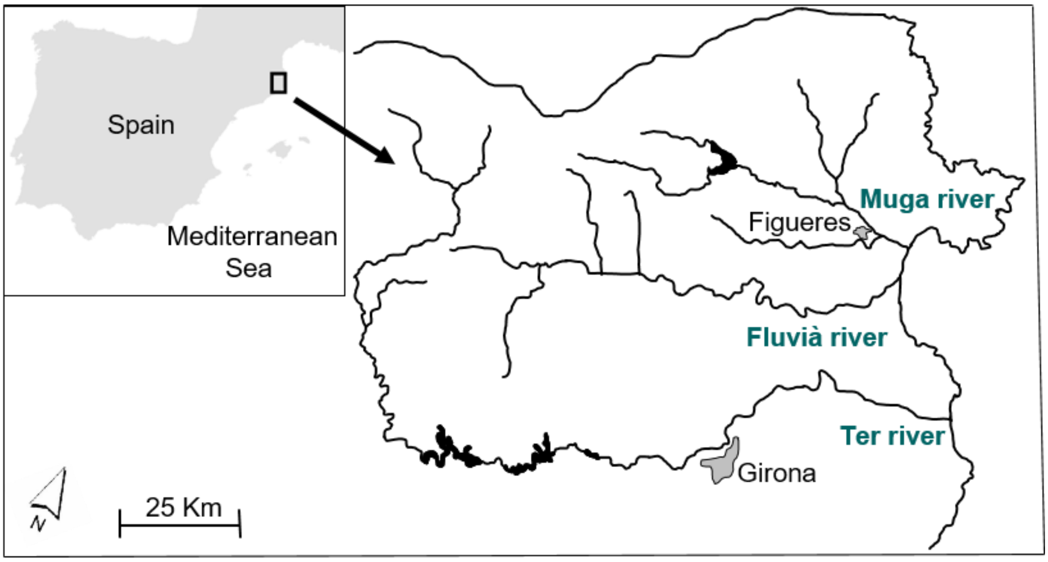
This section outlines the main experimental set-ups, as well as field and laboratory procedures. More specific information can be found on the Material and Method section within each chapter.

3.1 Aquatic respirometry

3.1.1 Fish sampling and holding conditions

A total of 8 species were collected between 2014 and 2017 for the assessment of fish swimming performance (Chapters I & II). Fish sampling was conducted by trained personnel using electrofishing techniques (backpack electroshocker: LR-24-Smith-Root Letd. 120 V DC-0.6 A) and dip nets (1-mm mesh size). The samples of bleak (*Alburnus alburnus*), Catalan chub (*Squalius laietanus*), roach (*Rutilus rutilus*), and pumpkinseed (*Lepomis gibbosus*) were collected from the Muga River (Figure 1); the sample of Mediterranean barbel (*Barbus meridionalis*) was collected from the Fluvià River (Figure 1), and the sample of minnow (*Phoxinus septimaniae*) from the Brugent River, a tributary of the Ter river (Figure 7). Spanish toothcarp (*Aphanius iberus*) was collected from La Rubina salt marshes in the Alt Empordà (42˚15’38.7’’ N, 3˚8’38.9’’ E). Moreover, data of mosquitofish (*Gambusia holbrooki*) were obtained from a previous study (Srean et al. 2017).

Fishes were held in rectangular tanks upon arrival at the University of Girona. Tanks were supplied with recirculated, filtered freshwater and vigorous aeration. Water changes were conducted twice a week in each tank to assist with maintaining water quality. A natural photoperiod cycle was used during the acclimation period. All fish were fed once a day with frozen bloodworms (*Chironomus sp.*) during the holding period, although feeding was interrupted for at least 24 hr prior to the experiment to avoid post-prandial metabolism. Experiments were always conducted between autumn and early spring to avoid the breeding seasons of species. After at least 2 weeks of being held under experimental conditions, fish were then individually transferred into the swim tunnel respirometers.



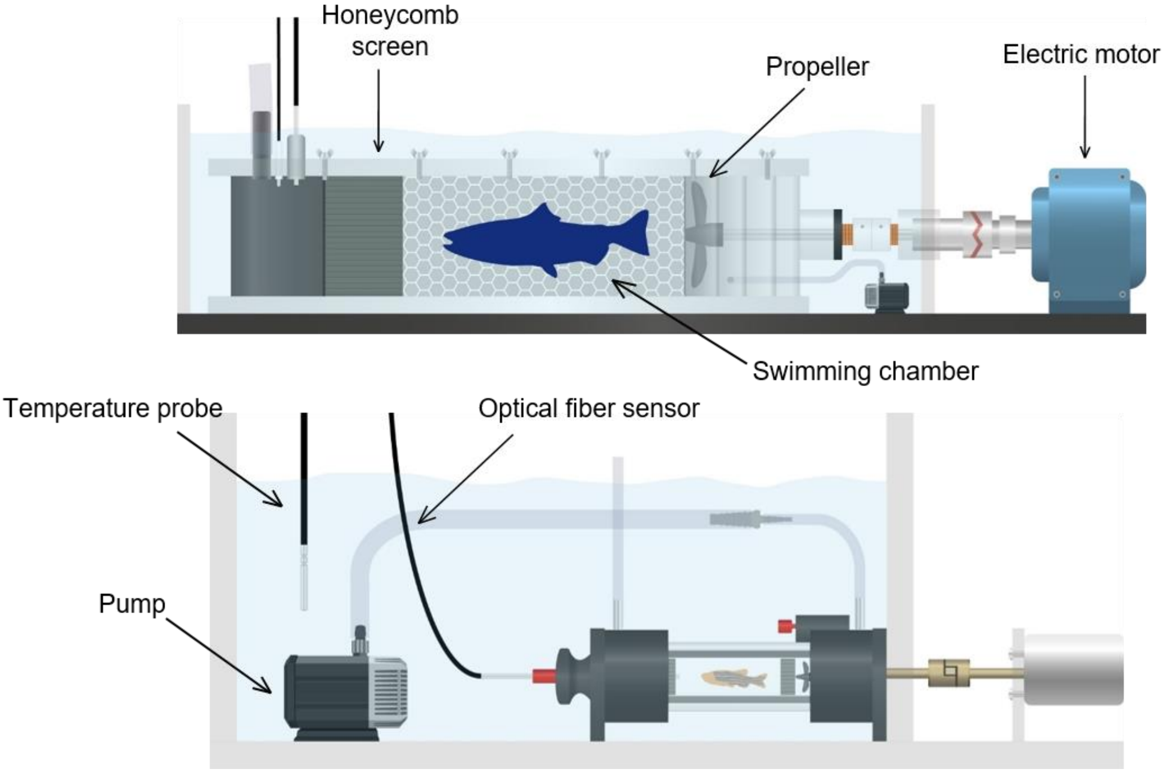
F I G U R E 1 Map of the Muga, Fluvià and Ter rivers, roughly corresponding with the province of Girona (Catalonia, Spain). The main cities and reservoirs (shaded areas) in the vicinity are also shown. Map courtesy of Lluís Benejam

3.1.2 Swim tunnel set-up

Swimming speeds and rates of oxygen consumption by fish were obtained using two types of Blazka-style swim tunnel respirometers (Loligo® Systems, Viborg, Denmark). The continuous laminar flow was generated by a propeller connected to the motor outside of the respirometers. Swim tunnels were connected to an optical fiber instrument (Witrox 1; Loligo® Systems, Tjele, Denmark) that allowed us to record dissolved oxygen concentration $mg \cdot L^{-1}$ at 1 Hz. Likewise, a temperature probe (Pt1000 temperature sensor; Witrox 1; Loligo® Systems) was used for the automated compensation of oxygen data to changes in temperature. Rates of oxygen consumption were measured using intermittent-flow respirometry, which is a series of short-term closed respirometry experiments, interrupted by flushing intervals to replace the respirometer water.

A large swim tunnel respirometer was used for cyprinids and pumpkinseed (Figure 2). The respirometer consisted of a rectangular measuring recirculation system equipped with a 5-L swimming chamber (30 cm length × 7.5 cm height ×7.5 cm width). An external water bath was connected to the respirometer and an Eheim pump flushed aerated water at a rate of 10 $\text{L}\cdot\text{min}^{-1}$ during the flush phase. We additionally connected the external water bath of the swim tunnel to a plastic supply tank containing 300 L of air-saturated water. An automated Eheim pump continuously provided freshwater from the supply tank to the external water bath and then water was recirculated again through a decantation system. The supply tank was equipped with an automated liquid cooler (85W, 972.46 BTU/h, J.P. Selecta®) to maintain the temperature at 20˚C.

A small swim tunnel respirometer was used for Spanish toothcarp and mosquitofish (Figure 2). The respirometer consisted of a 170-mL tubular swimming chamber (100 mm length × 26.4 mm internal diameter) immersed in an external water bath containing 25 L of clean, aerated water. An automated Eheim pump flushed aerated water at a rate of 5 $\text{L}\cdot\text{min}^{-1}$ between respirometer compartments during the flush phase.



F I G U R E 2 Schematic diagram of the swim tunnel respirometers used to test the swimming performance of fishes: 5-L swimming chamber (top) and 170-ml swimming chamber (bottom). Images obtained from: https://www.loligosystems.com

3.1.3 Determination of swimming performance and metabolic rates

Swimming performance

- Critical swimming speed (U_{crit}): A U_{crit} test was performed with step-wise increases in flow speed of approximately 1 BL·s^{-1} with a time interval of 20 min until the fish fatigued. We calculated U_{crit} according to Beamish (1978):

$$U_{crit} = U_f + U_i T_f T_i^{-1},$$

where U_f is the highest velocity maintained for the entire swimming period, U_i is the speed increment (cm·s^{-1}), T_f is the time elapsed at fatigue speed and T_i is the set interval time (min).

- Relative U_{crit} (BL·s^{-1}): Absolute U_{crit} values (cm·s^{-1}) were divided by standard length of fish.

Metabolic rates

- Oxygen consumption rates ($\dot{M}_{O_2}$): Regression slopes derived from the relationship between oxygen consumption in the respirometers and time were used to calculate oxygen consumption rates:

$$\dot{M}_{O_2} = -(\Delta Of - \Delta Ob) \times V \times 60,$$

where ΔOf and ΔOb are the slopes of the regression (mg O_2 l^{-1}·min^{-1}) due to fish respiration and microbial respiration, respectively, V is the volume of the respirometer (after subtracting the fish volume) and 60 was used to change units to mg O_2·h^{-1}.

- Maximal metabolic rate (MMR): Maximal metabolic rate (also termed 'maximum metabolic rate') was defined as the highest value of ($\dot{M}_{O_2}$) during the swimming trial.
- Standard metabolic rate (SMR): Exponential functions were fitted from the oxygen consumption rates ($\dot{M}_{O_2}$) and swimming speeds (*U*) according to Brett (1964), as follows:

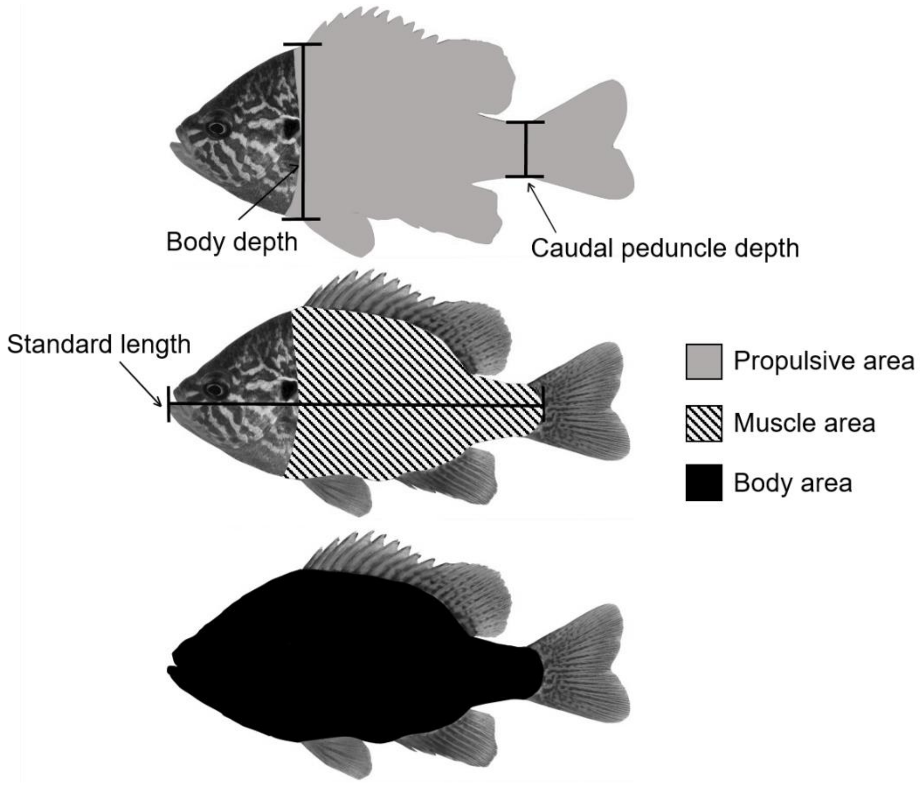
$$\dot{M}_{O_2} = a \times e^{bU},$$

where *a* is the estimated SMR at zero swimming speed and *b* an estimated constant that can be used as an index of swimming efficiency.

- Absolute aerobic scope (AAS): The difference between MMR and SMR.
- Mass-specific cost of transport (MCOT): The cost of transport was estimated by dividing the obtained $\dot{M}_{O_2}$ values by the corresponding speed (*U*) and body mass (Kg). MCOT values were then converted into energy units using an oxycaloric value of 14.1 J mgO_2^{-1} (Hepher, 1988).

3.1.4 Fish morphology

Following the swimming trials, individuals were weighed to the nearest 0.1mg and some morphological measurements (lengths and body areas) were obtained from digital images using tpsDig2 software (Rohlf, 2015). Some morphometric ratios were then calculated (Figure 3): fineness ratio (ratio between standard length and maximum body depth), caudal peduncle depth factor (ratio between caudal peduncle depth and maximum body depth), propulsive ratio (ratio between propulsive area and body area) and muscle ratio (ratio between muscle area and body area).



F I G U R E 3 Schematic diagram of the morphological measurements taken in fishes, including: standard length, maximum body depth (height at the deepest region), caudal peduncle depth (height at the narrowest region), body area (area in lateral view excluding fins), propulsive area (area including the fins (naturally fully extended) but excluding the head and the gut region where they are inflexible or lack overlaying muscle and cannot be used for propulsion), muscle area (area excluding the fins, the head and gut region)

3.2 Field mesocosm experiments

3.2.1 Study sites

The study sites where mesocosm experiments took place were situated in two tributaries of the Ter river (Figure 4): The Llémena Stream and the Osor Stream. The Ter river rises in the Pyrenees Mountains (at 2400 m a.s.l) and drains into the Mediterranean Sea. The Ter river is 208 km long and has a drainage area of 2955 km^2, with a main discharge of ca. 10 $m^3 s^{-1}$ (Benejam et al. 2012).

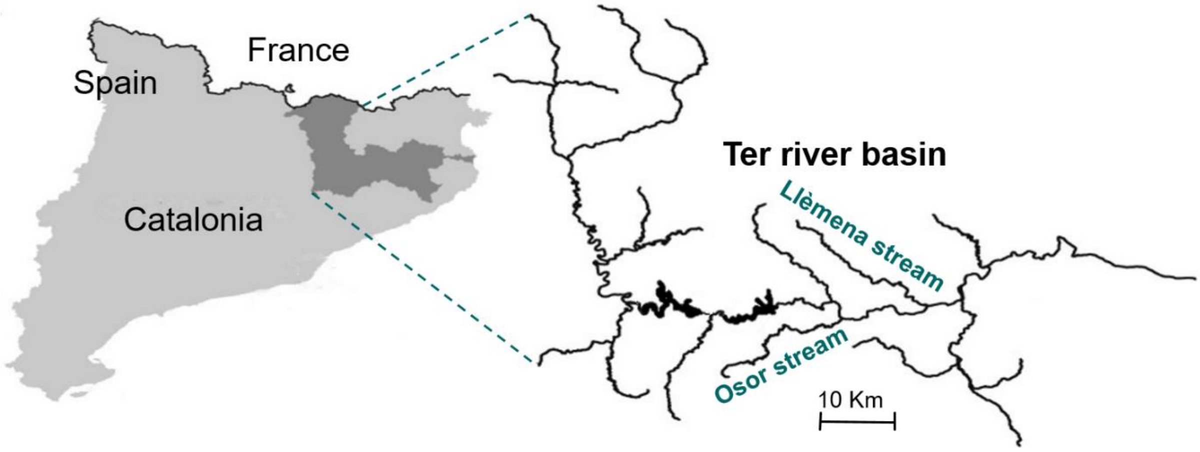


FIGURE 4 Location of the Llèmena and Osor streams within the Ter river basin

The Llémena stream

Llémena is a Mediterranean stream that rises in littoral mountains at 800 m a.s.l. It is a small calcareous stream with a length of ca. 32 km and a drainage area of 185 km^2 (Figure 4). This catchment is characterized by a typical coastal Mediterranean climate, where stream discharge largely depends on rainfall (annual average 700–900 mm year−1, Catalan Water Agency, ACA). Seasonal rainstorms causing floods occur mainly during the autumn and spring. In summer, stream discharge can be substantially reduced due to drought. According to the ACA, the mean discharge in the last 76 years has been of ca. 0.94 $m^3 s^{-1}$. The upper part of the stream is well preserved. However, human impacts such as eutrophication increases downstream due to agriculture, livestock, and urban activities (Argudo et al. 2020). Moreover, water diversion for irrigation (Figure 5) causes a decrease in instream flow that often leads to intermittency in summer.

The Osor Stream

The Osor Stream rises in the Guilleries Mountains at 1127 m a.s.l. It is a small siliceous stream with a length of ca. 24 km long and a drainage area of 88.9 km^2 (Figure 4). This stream is also subject to the Mediterranean climate with flow regimes reflecting precipitation patterns. In general, this mountain stream is well preserved, with a developed riparian vegetation, and low urban pressures. It receives small amounts of residual sewage from a wastewater treatment plant situated in St. Hilari Sacalm (5064 inhabitants) and from the Osor village (354 inhabitants). The stream is mainly affected by water diversion and continuous (mine effluent) and diffuse (mine run-off) metal inputs remaining from former mining activities (stopped laboring in 1980). The mine effluent is a canal draining the old underground mine galleries (Figure 5).

F I G U R E 5 The Llémena Stream (top) and the Osor Stream (bottom), with 2 of their main environmental stressors: (1) hydrological alteration caused by the presence of a weir for irrigation (on the top right corner), (2) and metal pollution caused by the mine effluent and mine run-off (on the bottom right corner). Note that the photograph taken in the Osor Stream shows one of the cages used for the mesocosm experiment

3.2.2 Experimental design

We set up mesocosm experiments with cages that allowed us to control fish density and quantify treatments effects on stream benthic communities. In the Llémena Stream (Chapter III), we used cages (1.2 × 1.2 × 0.7 m) made of 6-mm plastic mesh and supported by reinforcing steel bars. Whereas in the Osor Stream (Chapter IV), we considered to use four-sided cages (1.5 × 1 × 0.7 m) made of high-density polyethylene (4.5 mm-pore size) and supported by a wooden square structure (Figure 11). Thus, we avoid interference for the metal analysis that would otherwise occur with a steel structure. Treatments were established by varying fish densities in the cages, thus simulating either conditions of high or low fish densities, or the total disappearance of fish. For this purpose, we used the Mediterranean barbel (*Barbus meridionalis*) as a study case. This cyprinid fish is endemic in the Mediterranean streams of Spain and France (Doadrio et al. 2011). It is one of the smallest European species of the *Barbus* genus, attaining 270 mm (usually less than 200 mm) as a total length. This fish species is generally regarded as rheophilic and feeds mainly on benthic invertebrates. All treatments were replicated three times (3 cages per site/reach) and had a duration of 2 weeks. At the beginning of the experiment, cage bottoms were filled with stream cobbles to ensure a very close simulation of natural conditions and early colonization by stream communities.

3.2.3 Field sampling and laboratory procedures

Discharge and water characteristics

- Discharge ($m^3 s^{-1}$): In Chapter III, mean discharge was estimated using the salt dilution method (Elosegi and Sabater, 2009) that involves injecting an artificially tracer (NaCl) and determining its dilution, following complete mixing into the flow, by means of integration of the electrical conductivity (hand-held probe WTW, Weilheim, Germany) as a function of time. In Chapter IV, mean discharge was estimated using the water velocity (MiniAir2 vane anemometer, Schiltknecht) and the cross sectional area (Elosegi and Sabater, 2009).

- Physicochemical parameters: Water temperature (˚C), dissolved oxygen (mg L^{-1}), pH, electrical conductivity (µS cm^{-1}) were measured in situ using a multi-parametric probe (WTW Meters, Weilheim, Germany).
- Inorganic nutrients: Phosphate (µg $PO_4^{3-} \cdot L^{-1}$) and ammonium (µg $NH_4^+ \cdot L^{-1}$) concentrations were determined colorimetrically (Murphy and Riley, 1962; Reardon et al. 1966) using a spectrophotometer (Shimadzu UV-1800). In Chapter III, nitrate (µg $NO_3^- \cdot L^{-1}$) concentrations were measured using a specific kit (AQUANAL®-plus Nitrat (NO_3), FLuka), which allows to quantify nutrient concentrations within a range from 0,1 to 30 mg/L.
- Regarding the nutrient uptake experiment, dissolved nutrient concentrations were analysed using an Automatic Continuous Flow Futura-Alliance Analyser at the Nutrient Analysis Service of the CEAB-CSIC.
- Metals: Dissolved metals were determined after the acid digestion procedure using an inductively coupled plasma mass spectroscopy (ICP-MS 7500c Agilent Technologies) at the Research Technical Services of the University of Girona.

Periphyton

- Chlorophyll-*a* concentration (Chl-*a*): Chlorophyll-*a* was measured after extracting in 10 mL of 90% acetone for 24 h in the dark at 4 °C and sonicating for 2 min at 40 kHz. Chlorophyll-*a* concentration was determined spectrophotometrically (Jeffrey and Humphrey, 1975).
- Ash-free dry weight (AFDW): Samples were dried at 50 °C for 48 h, weighed, combusted at 450 °C for 4h, and reweighed to determine the ashes weight (AFDW).

- Nutrient content: samples were lyophilized for 24 h and homogenized using a mortar. Nitrogen (N) and carbon (C) content were measured using an elemental analyser (PerkinElmer 2400) at the Research Technical Services of the University of Girona. Phosphorus (P) content was determined after digestion following the protocol described by Murphy and Riley (1962).
- Metal concentrations: Digested samples were analysed by inductively coupled plasma mass spectroscopy (ICP-MS 7500c Agilent Technologies) at the Research Technical Services of the University of Girona.

Macroinvertebrates

- Community composition: Benthic macroinvertebrates were counted and identified to family under a dissecting microscope. Families of mayflies (Ephemeroptera), stoneflies (Plecoptera) and caddisflies (Trichoptera) were grouped as EPT taxa, whereas families of dragonflies (Odonata), beetles (Coleoptera) and true bugs (Hemiptera) were grouped as OCH taxa.
- Macroinvertebrates biomass: Samples were dried at 60 °C until constant mass and weighed (± 0.1 mg) to obtain the total dry mass (DM).
- Functional feeding groups (FFG): Following Tachet et al. (2000), macroinvertebrates were assigned to FFG, as follows: grazers, shredders, collector-gatherers (also termed ‘deposit-feeders’), collector-filterers and predators. FFG biomass and density were calculated by combining the biomass or density of each FFG in the sample. For taxa assigned to multiple FFGs, densities and biomasses were divided evenly into each applicable FFG (estimated as a percentage).
- Metal concentrations: Digested samples were analysed by inductively coupled plasma mass spectroscopy (ICP-MS 7500c Agilent Technologies). As for Zn, digested samples

were analysed by inductively coupled plasma mass (ICP-OES 5100 Agilent Technologies) at the Research Technical Services of the University of Girona.

Fishes

- Characterisation of fish assemblages: A four-person crew carried out electrofishing with multiple passes (three pass depletion) using a backpack electroshocker (LR-24-Smith-Root Letd. 120 V DC-0.6) in a 100-m reach for each sampling site: one person operating the anode, two people netting and one bringing a bucket. The individuals of each fish species were counted and abundances were estimated for each stream reach.

3.3 Statistical analyses

TABLE 1 Summary of the main statistical analyses used in each chapter

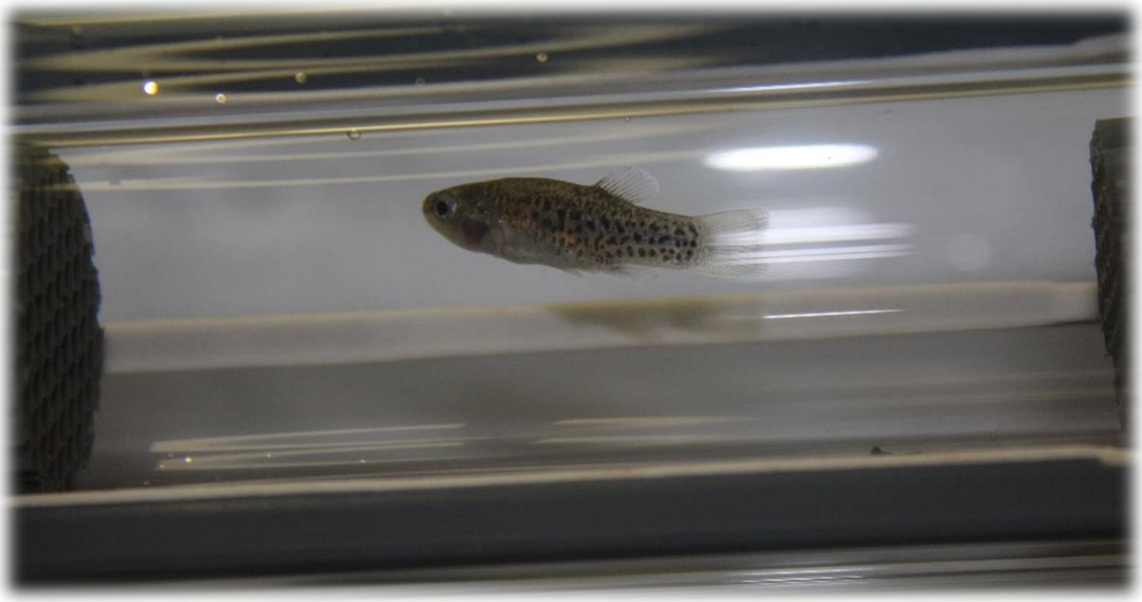
Statistical tool	Purpose of the analysis	Chapters
Simple linear regressions	Predicting swimming performance and metabolic traits as a function of fish size / Predicting metal concentrations in macroinvertebrates as a function of periphyton metal concentrations	I & II/ IV
Multiple linear regressions	Predicting swimming performance and metabolic traits as a function of several explanatory variables	II
Linear mixed models	Predicting the effects of ‘Reach’ and ‘Treatments’ on periphyton and macroinvertebrate variables, after accounting for random effects	IV
Pearson-product moment correlations	Association between response variables and morphometric ratios	I & II

Analysis of covariance (ANCOVA)	Variation in response variables between species and/or sexes, after accounting for fish size / Variation in metal concentrations among macroinvertebrates	I & II / IV
Analysis of variance (ANOVA)	Variation in response variables between species and sex, if variables were not related to fish size / Variation in response variables between reaches. If parametric assumptions were not met, Kruskal-Wallis test was then applied as an alternative to ANOVA	I / IV
Post hoc analysis	After ANOVA, Tukey and Dunn's tests of multiple comparisons were conducted to elucidate significant differences among groups	I & IV
Repeated-measures ANOVA	Variation in nutrient uptake by periphyton (over time) among reaches and treatments	III
Permutational analysis of variance (PERMANOVA)	Variation in response variables among reaches and treatments	III
Variation partitioning (VP)	Predicting individual fractions of variation explained by several predictors	II & III

4. RESULTS

CHAPTER I

Differences in swimming performance and energetic costs between an endangered native toothcarp (*Aphanius iberus*) and an invasive mosquitofish *Gambusia holbrooki*)



Rubio-Gracia F., Garcia-Berthou E., Latorre D., Moreno-Amich R., Srean P., Luo Y. and Vila-Gispert A. (2020). Differences in swimming performance and energetic costs between an endangered native toothcarp (*Aphanius iberus*) and an invasive mosquitofish (*Gambusia holbrooki*). *Ecology of Freshwater Fish, 29*, 230–240

Introduction

Swimming performance has long been seen as ecologically relevant for the determination of fitness and survival of species in aquatic ecosystems (Plaut, 2001). Predator-prey interactions (e.g. food capture, competition with other predators, and escapement from predators), reproduction and migration are processes that depend on the swimming ability of the individual (Fisher et al. 2000; Reidy et al. 2000). Similarly, energetic costs of swimming are also directly linked to several ecological processes (Videler, 1993; Webb, 1994), and consequently, the understanding of the energy budget of individuals have become increasingly important for developing bioenergetics models in ecological fish studies (e.g. Ohlberger et al. 2005).

In nature, steady swimming is commonly used by fish during foraging, reproduction, and seeking favourable environmental conditions (Plaut, 2001; Blake, 2004). When swimming faster, fish can reach critical swimming speed (U_{crit}), which is defined as the highest swimming speed that a fish can maintain for a time period (Brett, 1964). In general, U_{crit} is a standard measurement for evaluating the swimming capabilities of fishes (Plaut, 2001), and it involves aerobic and anaerobic metabolism (Videler, 1993; Hammer, 1995). Oxygen consumption measurements over time are usually used to estimate metabolic rates (Bell and Terhune, 1970). Two important metabolic traits can be estimated using the U_{crit} protocol, from which the respiratory capacity of a fish is generally described. Maximal metabolic rate (MMR) is traditionally defined as the maximum oxygen consumption rate during swimming exercise (Norin and Clark, 2016), whereas standard metabolic rate (SMR) is the baseline energy required for maintenance measured in post-absorptive, resting state (Chabot et al. 2016). Variation in SMR and MMR across species can be due to different growth strategies and contrasting lifestyles (Killen et al. 2010; Stoffels, 2015).

Based on fish locomotor theory, energetic costs of swimming are positively correlated to drag forces, which are determined by size or frontal area of a fish and swimming speed (Webb, 1975). Increased energetic costs are generally associated with stabilizing body posture and maintaining direction at lower speeds, and when fish face perturbing forces while swimming at higher speeds. However, there is a range of intermediate speeds at which fish can optimally swim. Thus, physiologists define optimal swimming speed (U_{opt}) as the speed at which the energetic cost of transport (COT) is the lowest (Tucker, 1970). Cost of transport and optimal swimming speed are commonly used to compare energetic costs between species (Videler, 1993; Ohlberger et al. 2006; Tudorache et al. 2008).

Body morphology is an important factor to be considered when determining swimming performance of fishes, because performance depends on drag forces and propulsion (Webb, 1984). Previous studies have explored the relationship between body morphology and swimming capacity among a wide range of fish species (Fisher and Hogan, 2007; Leavy and Bonner, 2009; Walker et al. 2013). Morphological traits that maximize steady swimming are an increased depth of the anterior body and head, a shallow caudal region and a streamlined body shape (Fisher and Hogan, 2007; Walker et al. 2013). In particular, a streamlined or fusiform body shape can also influence energetic costs of swimming due to a decrease of drag pressures along the body and a reduction of recoil energy losses (Webb, 1975; Scarnecchia, 1988; Langerhans, 2009; Langerhans and Reznick, 2010). Therefore, it is generally assumed that fishes with different morphological traits would display variation in swimming performance and energy metabolism (Pettersson and Bronmark, 1997).

The present study compares the swimming capacity and metabolic rates of the Spanish toothcarp, *Aphanius iberus* (Valenciennes, 1846), and the eastern mosquitofish *Gambusia holbrooki* Girard, 1859, in relation to their body shape. Spanish toothcarp (*A. iberus*, hereinafter) is an endemic cyprinodontiform fish of the Iberian Peninsula, whose current populations are restricted to a few isolated areas in salt marshes, coastal lagoons and river mouths along the Spanish Mediterranean coastline (Oliva-Paterna et al. 2006; Alcaraz and

García-Berthou, 2007a). Human pressure, water pollution, habitat fragmentation and, especially, the presence of the invasive mosquitofish (*G. holbrooki*, hereinafter) are some of the most pressing threats for its conservation (Oliva-Paterna et al. 2006). So far, some research has been focused on assessing ecological and behavioural aspects that could mediate the interspecific competition between these two species (Rincón et al. 2002; Alcaraz et al. 2008a; Carmona-Catot et al. 2013; Magellan and García-Berthou, 2016). In relation to swimming capabilities and metabolism, a previous study reported data on swimming performance and metabolic traits of *G. holbrooki* (Srean et al. 2016), yet little is known about *A. iberus* on this issue. As with the invasive competitor, *A. iberus* is a so-called 'limnophilic' species, i.e. preferring standing waters, so it may be of interest to compare their physiological response to constant water flow.

The main objectives of this study were to (1) determine prolonged swimming speeds and metabolic traits of *A. iberus* and (2) compare swimming performance and energy use between *A. iberus* and *G. holbrooki* in relation to morphological aspects. We hypothesized that: (i) *A. iberus* would display lower swimming speeds and higher cost of transport than *G. holbrooki,* given the differences in habitat use and migration rates between these two species (Alemadi and Jenkins, 2008; Díez-del-Molino et al. 2018); and (ii) differences in swimming capacity and efficiency would be determined by their morphological attributes, because of its intimate relationship with swimming ability and kinematics (Leavy and Bonner, 2009).

Material and methods

Study area and experimental fish

Specimens of *A. iberus* were collected using a dip net from La Rubina salt marshes in Alt Empordà, north-eastern Iberian Peninsula (42°15′38.7″ N, 3°8′38.9″ E) in 2017. Further information of the sampling area can be found elsewhere (García-Berthou and Moreno-Amich, 1992). Animal research and fieldwork were authorized by the Autonomous Government of Catalonia (Expedient PNAE 2017PNATAAEAUT075), the Commission of Animal Experimentation (Ref.: CEA-OH/9673/1) and by the Aiguamolls de l'Empordà Natural Park. Sampling was performed until obtaining up to twenty individuals of each sex. In total, thirteen females (20–44 mm total length and 70–1400 mg fish mass) and fifteen males (20–40 mm total length and 40–750 mg fish mass) of *A. iberus* were used for the comparison with 30 females and 30 males of *G. holbrooki* (15-43 mm total length and 40-650 mg fish mass*;* 21-34 mm total length and 80-370 mg fish mass, females and males, respectively). After sampling, fish were immediately transported to the laboratory, where they were acclimated to experimental conditions for 2 weeks prior to the experiment. Fish were placed into glass aquariums (90 l) at a temperature of 25 ± 1°C under a natural photoperiod cycle. Vigorous aeration was provided to all aquariums ensuring at least 90% of air saturation. From the second day of acclimation, fish were fed once a day with frozen bloodworms (*Chironomus* sp.) to ensure that most individuals had begun feeding. Feeding was interrupted for 24 h prior to the experiment to avoid postprandial effects during experiment. No mortalities occurred during the acclimation period, and visually all fish remained in a good health. The experiment carried out in this study were conducted in early spring to avoid the breeding season of this fish species (García-Berthou and Moreno-Amich, 1992; Oliva-Paterna et al. 2006). After swimming performance trials, fish were kept in a quarantine tank for 1 week and were returned to the same place where they were caught.

Determination of swimming performance and metabolic traits

Data on swimming capacity and metabolism for *G. holbrooki* were obtained from our previous study (Srean et al. 2016), where details on the experimental fish and methods can be found. As for *A. iberus*, we used essentially the same methodology in order to ensure that both species were tested under the same experimental conditions. Briefly, respirometry was conducted using a modified Bläzka-type swimming tunnel, with a non-turbulent laminar flow and equipped with a continuous-flow respirometer (Loligo® Systems, Viborg, Denmark). Flow was generated by a propeller connected to the motor outside of the respirometer. The tubular test section (170 ml volume, 100 mm length × 26.4 mm internal diameter) was laterally covered with non-reflecting white screens to avoid disturbing fish by 'mirror effects'. An external buffer tank was connected to the respirometer, and an automated flush pump flushed the air-saturated water at a rate of 5 $\mathrm{l \cdot min^{-1}}$ inside the respirometer to ensure well-mixed water. The temperature in the swim tunnel was kept constant at 25 ± 1°C by using a heater (Eheim Jäger Model 3613; EHEIM GmbH & Co KG, Deizisau, Germany) placed in the buffer tank.

Rate of oxygen consumption ($\dot{M}_{O_2}$) was measured using computerized, intermittent-flow respirometry. The respirometer was periodically flushed with aerated water for 2 min followed by a 1 min closed mixing period and then 20 min of closed respirometry. Measurements of oxygen consumption were recorded for a period of 20 min due to it has been shown to be sufficient to detect changes in dissolved oxygen concentration (Plaut, 2000). An optical fibre oxygen instrument (Witrox 1; Loligo® Systems, Tjele, Denmark) was used to measure dissolved oxygen concentration in water. Atmospheric pressure on the oxygen probe was adjusted based on the actual pressures before the oxygen consumption measurement. For calibration purposes, two-point calibration with the oxygen sensor was used to record the highest concentration value as 100% air-saturated and the lowest concentration value as 0% using a solution of sodium sulphite (Na_2SO_3, 0.159 M).

Fish were placed into the respirometer and allowed to acclimatise for 1 hour to an initial velocity of ca. 0.5 $\mathrm{BL \cdot s^{-1}}$ (body length, taken as the standard length of the fish, per second). After that, a critical swimming speed (U_{crit}) test was performed with step-wise increases in flow speed of approximately 1 $\mathrm{BL \cdot s^{-1}}$ until fatigue. Fatigue was defined as occurring when a fish could no longer swim against the current continuously (Farrell, 2008). Following Brett (1964), the critical swimming speed (U_{crit}, cm·s^{-1}) was calculated as:

$$U_{crit} = U_f + U_i T_f T_i^{-1},$$

where U_f is the highest speed maintained for a full time period (in cm·s^{-1}), T_f is the time swum at the last speed increment (in min), T_i is the set interval time (i.e. 20 min), and U_i is the speed increment (in cm·s^{-1}). Swimming speeds were not corrected by the 'solid-blocking effect' because the cross-sectional area of the fish never exceeded 10% of that of the respirometer (Bell and Terhune, 1970). Background microbial respiration inside the respirometer was calculated with no fish for 10 min at the end of each trial (i.e. blank run) to correct metabolic rates.

Measurements of oxygen consumption of individual fish were recorded during the U_{crit} swimming test. Oxygen consumption was calculated by fitting linear regression of the oxygen concentration decline over time. The resulting slope or regression coefficient was used to calculate oxygen consumption rates ($\dot{M}_{\mathrm{O_2}}$, mg O$_2$·h^{-1}):

$$\dot{M}_{\mathrm{O_2}} = -(\Delta Of - \Delta Ob) \times V,$$

where ΔOf *and* ΔOb are the oxygen consumption rates (mg O$_2$ l^{-1}·min^{-1}) due to fish respiration and microbial respiration, respectively, and V is the volume of the respirometer (after subtracting the fish volume). $\dot{M}_{\mathrm{O_2}}$ were then expressed in mg O$_2$·h^{-1}. The exponential function was used to describe the relationship between $\dot{M}_{\mathrm{O_2}}$ (log-transformed) and swimming speed through linear regression (Brett, 1964; Webb, 1975; Beamish, 1978; Pettersson and Hedenström, 2000; Korsmeyer et al. 2002; Tudorache et al. 2008):

$$\dot{M}_{\mathrm{O_2}} = \mathrm{SMR} \times e^{bU},$$

where SMR is the standard metabolic rate extrapolated to zero activity and b, an estimated constant that can be used as an index of the swimming efficiency. The higher the value of b, the more marked the increase in the swimming $\dot{M}_{\mathrm{O_2}}$ values with an increased swimming speed.

In addition to SMR, two metabolic traits were obtained from the measurement of $\dot{M}_{\mathrm{O_2}}$: maximal metabolic rate (MMR) was determined as the highest value of $\dot{M}_{\mathrm{O_2}}$ during the U_{crit} swimming test, which was usually close to the fastest speed (Keen and Farrell, 1994; Nelson, 2016); and absolute aerobic scope (AAS) was calculated as the difference between MMR and SMR as an indicator of the physiological capacity of fish to perform several oxygen-demanding processes simultaneously (Clark et al. 2013).

Determination of fish swimming efficiency

The optimal swimming speed (U_{opt}, cm·s^{-1}), i.e., the swimming speed with the lowest energy cost per unit distance, was calculated from the exponential function according to Pettersson and Hedenström (2000):

$$U_{opt} = \frac{1}{b}$$

Consequently, the minimum cost of transport (COT, J·m^{-1}) was estimated as:

$$\mathrm{COT} = \frac{\dot{M}_{\mathrm{O_{2opt}}}}{U_{\mathrm{opt}}}$$

where $\dot{M}_{\mathrm{O_{2opt}}}$ is the oxygen consumption rate obtained at U_{opt}. In addition, the relationship between COT and swimming speed was evaluated by dividing the obtained $\dot{M}_{\mathrm{O_2}}$values by the corresponding speed. COT values were then converted into energy units using an oxycaloric value of 14.1 J·mg $\mathrm{O_2}^{-1}$ (Hepher, 1988) and corrected for size effects by dividing with fish mass. Mass-specific cost of transport (MCOT) was expressed in J·m^{-1}·kg^{-1}.

Fish morphology

After the experimental procedure, each individual was measured to the nearest (± 1 mm) and weighed to the nearest (± 0.1 mg). To analyse morphological traits, some morphometric measurements were obtained from digital images of the individuals. These measurements included standard body length, total body length, maximum body depth and least depth of caudal peduncle. Two morphometric ratios were then obtained from these measurements: fineness ratio (ratio between standard body length and maximum body depth) and caudal peduncle depth factor (ratio between least depth of caudal peduncle and maximum body depth). Both morphological ratios have been previously used as predictors of swimming ability and efficiency (Webb, 1975; Scarnecchia, 1988; Fisher and Hogan, 2007; Ohlberger et al. 2006; Walker et al. 2013).

Statistical analyses

All statistical analyses were performed with the R software (R Core Team, 2018). Analysis of covariance (two-way ANCOVA) was used to test for differences in swimming performance and energetic costs between species and sexes as categorical factors. Fish mass was the best predictor of all metrics (i.e. r^2 values were always higher for mass than for total body length), and was thus used as covariate for further analyses. Additionally, swimming speed was used as covariate to test for differences in MCOT between species. The assumption of parallelism of standard ANCOVA was tested analysing the interactions between the covariate and the categorical factors. If such interactions are not significant, they indicate that the slopes are homogeneous and the assumption of parallelism is thus satisfied. Then, the interactions were removed from the model in order to improve the statistical power of the ANCOVA (García-Berthou and Moreno-Amich, 1993). Afterwards, if the interaction between species and sex was found significant, ANCOVA was separately performed for each species to elucidate the differences between sexes within each species. Finally, the effects of sex were tested within each species when the assumption of parallelism was not satisfied. Consequently, if slopes were homogeneous and differences between sexes were not significant, ANCOVA was then applied to test for differences between species (both sexes pooled).

When response variables were not correlated to fish size, an analysis of variance (two-way ANOVA) was performed to test for differences between species and sex. After that, a Tukey's multiple comparison test (Tukey's HSD) was conducted to compare the means of the four groups (i.e. species by sex). Finally, relations between morphological traits and critical swimming capacity (U_{crit}) and swimming efficiency (U_{opt} and COT) were evaluated from Pearson product-moment correlation coefficients (r). All variables were $\log_{10}$-transformed to satisfy the assumptions of the parametric statistical methods (i.e. normality, homoscedasticity, and linearity).

Results

Swimming performance and metabolic traits

The interactions between fish mass and the categorical factors (i.e. species and sex) in the ANCOVAs were not significant for U_{crit}, MMR and AAS ($P > 0.05$), and hence homogeneous slopes among groups were assumed. U_{crit}, MMR and AAS were significantly different between the two species (Table 1), with marked differences after accounting for fish mass (i.e. different intercepts or adjusted means) (Table 2). *G. holbrooki* had higher swimming capacity (Figure 1a). Values of U_{crit} for the 28 *A. iberus* ranged from 5.04 to 18.90 $\mathrm{cm \cdot s^{-1}}$, while for the 60 *G. holbrooki* ranged from 4.85 to 22.26 $\mathrm{cm \cdot s^{-1}}$. Concerning metabolic traits, *A. iberus* had higher MMR (Figure 1b) and AAS (Figure 1c) compared to *G. holbrooki*. Values of MMR and AAS for *A. iberus* ranged from 0.10 to 1.21 mg $O_2 \cdot h^{-1}$ and from 0.06 to 0.52 mg $O_2 \cdot h^{-1}$, respectively. As for *G. holbrooki*, MMR and AAS ranged from 0.05 to 0.55 mg O_2 h^{-1} and from 0.03 to 0.40 mg $O_2 \cdot h^{-1}$, respectively. The species × sex interaction was found to be significant for U_{crit}, MMR and AAS (Table 1), thereby showing that differences among sexes depend on species.

Males of *G. holbrooki* had higher U_{crit} ($F_{1,\ 57} = 20.51$, $P < 0.001$), MMR ($F_{1,\ 57} = 5.62$, $P = 0.021$) and AAS ($F_{1,\ 57} = 5.59$, $P = 0.022$) than females, whereas sexes of *A. iberus* had similar U_{crit} ($F_{1,\ 25} = 0.002$, $P = 0.965$), MMR ($F_{1,\ 25} = 2.86$, $P = 0.104$) and AAS ($F_{1,\ 25} = 1.77$, $P = 0.195$), after accounting for the effects of fish mass.

The assumption of parallelism was not satisfied for SMR, and therefore the effects of sex were tested within each species. There were no differences in SMR between sexes in *A. iberus* ($F_{1,\ 25} = 2.33$, $P = 0.140$) and *G. holbrooki* ($F_{1,\ 56} = 0.13$, $P = 0.718$). SMR was not significantly different between species (Table 1, Figure S1).

TABLE 1 Analyses of covariance (ANCOVA) of swimming performance and energetic costs for Spanish toothcarp (*Aphanius iberus*) ($N = 28$) and mosquitofish (*Gambusia holbrooki*) ($N = 60$)

	U_{crit}			MMR			AAS			SMR			COT		
	$R^2_{\mathrm{adj}} = 0.607$			$R^2_{\mathrm{adj}} = 0.579$			$R^2_{\mathrm{adj}} = 0.444$			$R^2_{\mathrm{adj}} = 0.238$			$R^2_{\mathrm{adj}} = 0.603$		
	SS	df	*P*	SS	df	*P*	SS	df	*P*	SS	df	*P*	SS	df	*P*
M	0.570	1	***	1.605	1	***	1.369	1	***	2.678	1	***	0.615	1	***
Species	0.372	1	***	0.773	1	***	0.808	1	***	0.207	1	0.193	3.085	1	***
Sex	0.000	1	0.954	0.109	1	0.052	0.046	1	0.293	-	-	-	-	-	-
Species × Sex	0.090	1	**	0.159	1	*	0.178	1	*	-	-	-	-	-	-
Residuals	0.978	83		2.295	83		3.342	81		10.10	84		2.909	72	

Note: Models test differences between species and sexes for U_{crit}, MMR and AAS with fish mass (*M*) as a covariate. Models test differences between species for SMR and COT with fish mass as a covariate. The assumption of parallelism was not satisfied for U_{opt} (optimal swimming speed), and therefore ANCOVA was not applied. All variables were $\log_{10}$-transformed ($*P \leq 0.05$, $**P \leq 0.01$, and $***P \leq 0.001$). Abbreviations: AAS, absolute aerobic scope; COT, minimum cost of transport; MMR, maximal metabolic rate; SMR, standard metabolic rate; U_{crit}, critical swimming speed

TABLE 2 Linear regression functions ($y = a + bx$) of the swimming performance and energetic costs with fish mass for Spanish toothcarp (*Aphanius iberus*) and mosquitofish (*Gambusia holbrooki*)

Species	Sex	Independent variable	a	95% CI	b	95% CI	R^2_{adj}	N	P
A. iberus	Pooled	U_{crit}	1.078	(0.990, 1.166)	0.291	(0.163, 0.419)	0.434	28	***
G. holbrooki	Female	U_{crit}	1.282	(1.189, 1.375)	0.288	(0.158, 0.417)	0.404	30	***
G. holbrooki	Male	U_{crit}	1.410	(1.172, 1.648)	0.279	(0.008, 0.550)	0.107	30	*
A. iberus	Pooled	MMR	-0.199	(-0.376, -0.023)	0.390	(0.132, 0.648)	0.243	28	**
G. holbrooki	Female	MMR	-0.290	(-0.403, -0.176)	0.612	(0.453, 0.771)	0.679	30	***
G. holbrooki	Male	MMR	-0.209	(-0.489, 0.072)	0.606	(0.287, 0.926)	0.328	30	**
A. iberus	Pooled	AAS	-0.431	(-0.580, -0.281	0.378	(0.159, 0.597)	0.300	28	**
G. holbrooki	Female	AAS	-0.559	(-0.760, -0.358)	0.593	(0.297, 0.889)	0.363	30	***
G. holbrooki	Male	AAS	-0.377	(-0.826, 0.073)	0.636	(0.127, 1.145)	0.166	30	*
A. iberus	Pooled	SMR	-0.604	(-0.956, -0.251)	0.595	(0.080, 1.111)	0.146	28	*
G. holbrooki	Pooled	SMR	-0.718	(-0.933, -0.502)	0.585	(0.314, 0.856)	0.233	60	***
A. iberus	Pooled	U_{opt}	1.151	(0.967, 1.336)	0.393	(0.123, 0.663)	0.228	28	**
G. holbrooki	Pooled	U_{opt}	1.376	(1.262, 1.490)	0.335	(0.195, 0.476)	0.292	60	***
A. iberus	Pooled	COT	-1.728	(-1.929, -1.526)	0.202	(-0.093, 0.497)	0.035	28	0.17
G. holbrooki	Pooled	COT	-2.020	(-2.192, -1.848)	0.378	(0.162, 0.594)	0.20	60	***

Note: A single linear regression function is shown when differences between sexes were not significant ($p > .05$). All variables were $\log_{10}$-transformed (*$P \leq 0.05$, **$P \leq 0.01$, and ***$P \leq 0.001$). Abbreviations: AAS, absolute aerobic scope; COT, minimum cost of transport; MMR, maximal metabolic rate; SMR, standard metabolic rate; U_{crit}, critical swimming speed; U_{opt}; optimal swimming speed

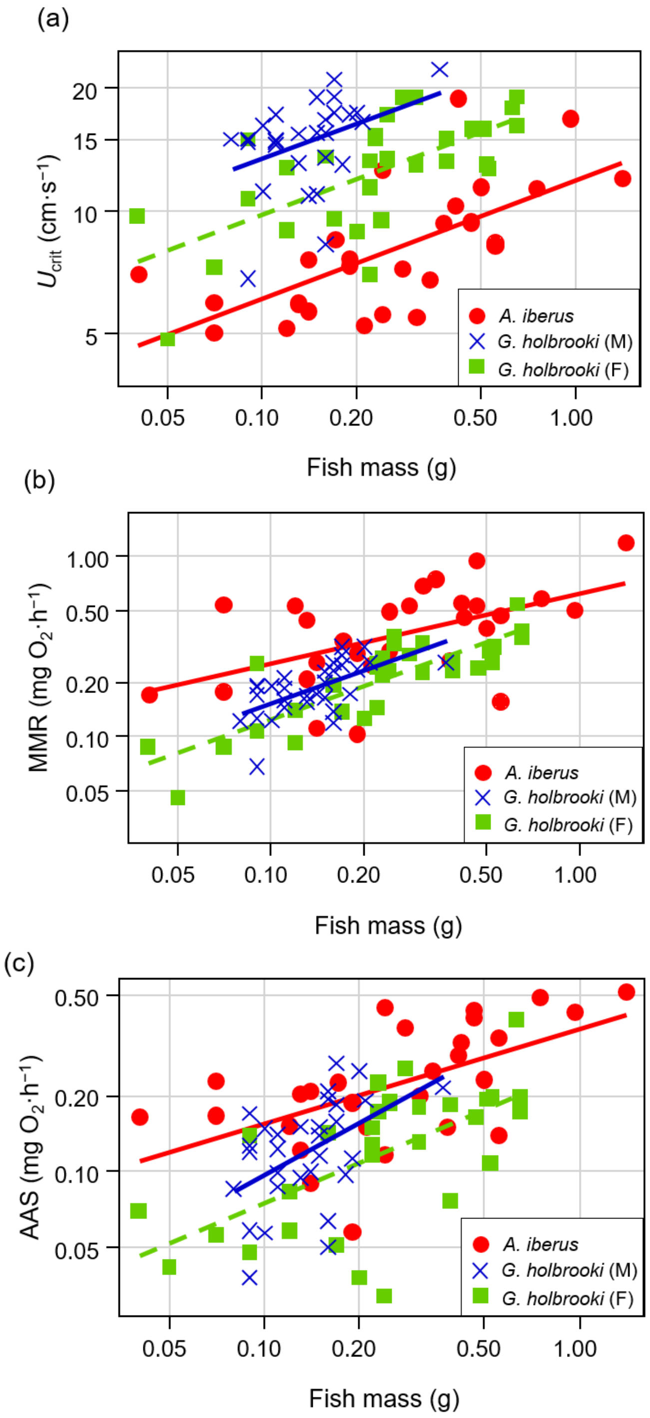


FIGURE 1 Relationships of critical swimming speed U_{crit} (a), maximal metabolic rate MMR (b) and absolute aerobic scope AAS (c) with fish mass for Spanish toothcarp, *Aphanius iberus* (N = 28) and mosquitofish, *Gambusia holbrooki* (N = 60). Regressions are significantly different between species (red circles for both sexes of *A. iberus*) and between sexes of mosquitofish (green squares for females and blue multiplication signs for males) for U_{crit}, MMR and AAS (Table 1). For clarity, regression of females of *G. holbrooki* is represented by a dashed line. Regression coefficients are shown in Table 2. Note both axes are on a log scale

Swimming efficiency

The effects of sex were also tested for U_{opt} and COT within each species. There were no differences in COT between sexes of *A. iberus* ($F_{1,\,25} = 3.38$, $P = 0.078$) and between sexes of *G. holbrooki* ($F_{1,\,45} = 0.43$, $P = 0.517$). ANCOVA showed significant differences in COT between species (Table 1). *A. iberus* generally displayed higher COT (Figure 2a) compared to *G. holbrooki*, for a given body mass. Within each species, the slope (*b*) of COT was found to be higher in *G. holbrooki* (Table 2), although *A. iberus* generally displayed more variability (i.e. a wider 95% CI). COT ranged from 0.004 to 0.034 $\mathrm{J \cdot m^{-1}}$ and from 0.001 to 0.012 $\mathrm{J \cdot m^{-1}}$ in *A. iberus* and *G. holbrooki*, respectively. In addition, MCOT was negatively related with swimming speed ($P < 0.001$), suggesting that both species swam more efficiently with increasing swimming speed. Most individuals of *A. iberus* generally had higher MCOT than *G. holbrooki* as a function of swimming speed (Figure 2b). ANCOVA showed interaction between swimming speed and species ($P = 0.014$), indicating thus that variation in the energetic cost in relation to swimming speed was different between species ($F_{1,\,483} = 21.73$, $P < 0.001$). As for U_{opt}, the assumption of parallelism was not satisfied, i.e. slopes were not homogeneous between sexes of *G. holbrooki*, and therefore a ANCOVA was not applied. *A. iberus* generally displayed lower U_{opt} than *G. holbrooki* (Figure S2).

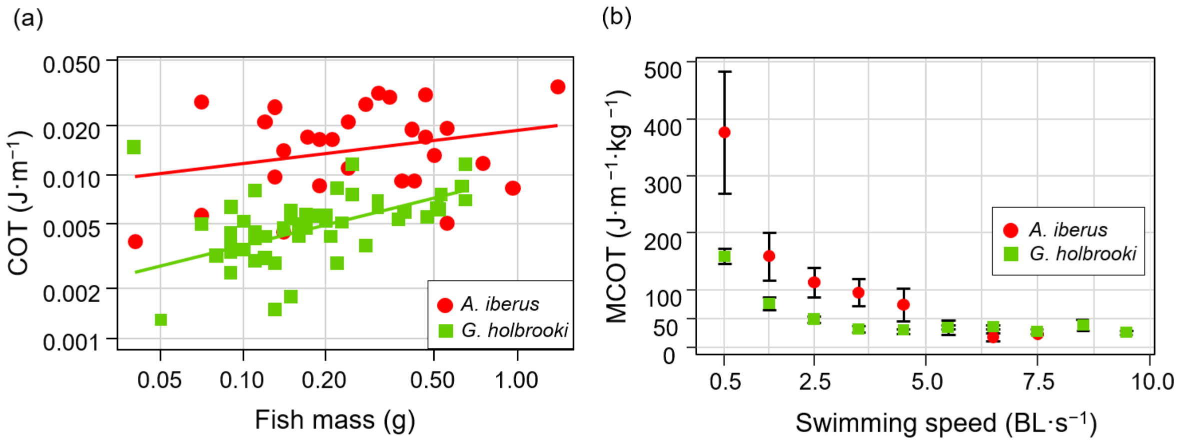
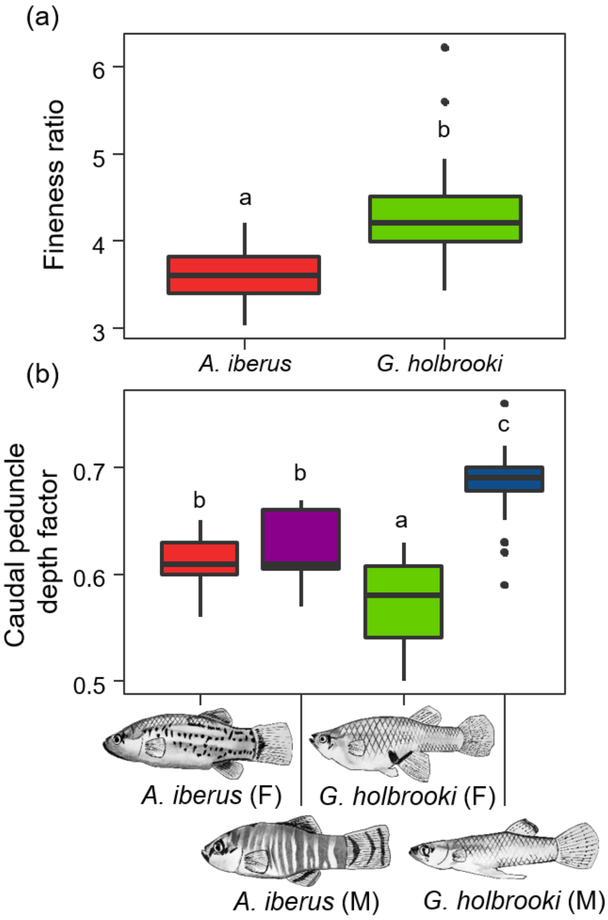


FIGURE 2 Relationships of minimum cost of transport COT with fish mass (a) and mass-specific cost of transport MCOT with swimming speed (b) for Spanish toothcarp, *Aphanius iberus* (N = 28) and mosquitofish, *Gambusia holbrooki* (N = 60). COT was significantly diferent between Spanish toothcarp and mosquitofish (Table 1). Regression coefficients are shown in Table 2. MCOT was derived from the swimming speed and metabolic rates (mean ± SE). Note both axes in Figure 2a and y-axis in Figure 2b are on a log scale

Relationship between fish morphology and swimming efficiency

Morphological ratios were not significantly related to fish size. ANOVA showed significant differences in fineness ratio between species ($F_{1,\,77} = 55.19$, $P < 0.001$). When accounting for both sexes, *G. holbrooki* had higher values of fineness ratio than *A. iberus* (Tukey test, $P < 0.05$; Figure 3a). In addition, we found significant positive correlations of fineness ratio with U_{crit} ($r = 0.39$, df = 79, $P < 0.001$) and U_{opt} ($r = 0.29$, df = 76, $P < 0.01$). Instead, fineness ratio had a significant negative correlation with COT ($r = -0.45$, df = 72, $P < 0.001$). As for the caudal peduncle depth factor, sex as factor ($F_{1,\,78} = 101.61$, $P < 0.001$) and the species × sex interaction ($F_{1,\,78} = 38.27$, $P < 0.001$) were significant; males of *G. holbrooki* had the highest values, followed by *A. iberus* (without differences between sexes) and finally, females of *G. holbrooki* showing the lowest values (Tukey test, $P < 0.05$; Figure 3b). In addition, caudal peduncle depth factor was not significantly correlated with U_{crit} ($r = 0.11$, df = 80, $P = 0.307$)

and U_{opt} (r = –0.05, df = 77, P = 0.693). However, we found a significant negative correlation of caudal peduncle depth factor with COT (r = –0.24, df = 73, P = 0.041).



F I G U R E 3 Fineness ratio (a) and caudal peduncle depth factor (b) for Spanish toothcarp, *Aphanius iberus* and mosquitofish, *Gambusia holbrooki*. Boxplots show median (line), quartiles (box) and ranges excluding outliers (error bars). Significant difference represented by different lowercase letter from Tukey's HSD ($p < .05$)

Discussion

The present study reports the first data on the swimming performance and energy metabolism of the endangered Spanish toothcarp (*A. iberus*). Concerning swimming speeds, *A. iberus* displayed low critical swimming capacity reaching the fatigue levels nearby 3–5 BL·s^{-1} (U_{crit} mean = 8.64 cm·s^{-1}). This swimming capacity is far below the average of similar small-bodied species (Plaut, 2002; Li et al. 2017), and other cyprinodontiforms (Plaut, 2000). Swimming capacity and metabolic rates increased significantly with fish mass in *A. iberus*, showing that heavier fish performed best. Additionally, we investigated the effects of sex on swimming capacity and metabolism of this native toothcarp. Results suggest that both sexes present a similar steady locomotor performance and energy use, since no differences were detected for any of the response variables studied. In general, species that present a distinct sexual dimorphism are expected to display differences in swimming performance between sexes (Royle et al. 2006; Oufiero and Garland, 2007; Conradsen and McGuigan, 2015). However, that is not likely the case as for *A. iberus* despite the fact that females are generally larger than males. Even though we did not measure specific body traits in *A. iberus*, the lack of differences in some morphometric ratios (i.e. caudal peduncle depth factor and fineness ratio) might not lead to sex-dependent differences in swimming performance. In contrast, the effects of sex in *G. holbrooki* are more clear since males displayed higher critical swimming speeds and maximal metabolic rates than females, as previously observed in Srean et al. (2016). However, while Srean et al. (2016) did not find significant differences in the factorial aerobic scope (FAS) between sexes, in this study males had higher absolute aerobic scope than females. This finding is not surprising since calculations of factorial aerobic scope (i.e. MMR/SMR) could be strongly influenced by variation in the ratio's denominator, whereas absolute aerobic scope (i.e. MMR-SMR) is generally less sensitive to variation in standard metabolism among individuals (Clark et al. 2013; Halsey et al. 2018).

We observed large differences in swimming performance and metabolism between *A. iberus* and *G. holbrooki*, being the invasive species which swam faster (U_{crit} mean = 14.11 $\mathrm{cm \cdot s^{-1}}$). However, this swimming capacity can still be considered low, especially if compared to rheophilic species (Tudorache et al. 2008). Despite this, the reduced swimming capacity of *G. holbrooki* has not stopped it from living also in downstream rivers and streams (Benejam et al. 2009; Carmona-Catot et al. 2011), where water velocity is much reduced (Díez-del-Molino et al. 2018). In fact, the invasion success of certain species is not always related to a high steady swimming capacity (e.g. Tierney et al. 2011). On the other hand, the endangered *A. iberus* is mainly found in calm waters (Alcaraz et al. 2008b), and recent research (Gonzalez et al. 2018) pointed out that habitat fragmentation appears to reduce the migration of individuals causing increased genetic drift across its geographical range. So, the preference for slow-moving habitats agrees with the poor swimming capacity shown by this fish species. In fact, there is a close link between habitat characteristics (e.g. water flow) and swimming performance of a fish (Langerhans, 2009; Alexandre et al. 2014; Oufiero and Whitlow, 2016).

After accounting for fish size, the minimum cost of transport was more favourable in *G. holbrooki*, since it displayed less energy-consumption per meter swum. This means that Spanish toothcarp used distinctly more energy than mosquitofish of the same mass swimming at the same speed. In addition, when comparing energetic costs at different swimming speeds, differences between species are also remarkable. We found evidence that *A. iberus* showed high mass-specific cost of transport at lower speeds due to high metabolism required for locomotion, obtaining thus poorer swimming efficiency. In contrast, *G. holbrooki* showed similar mass-specific cost of transport in a wider range of swimming speeds, indicating that this species can optimally swim without excessively increasing the energetic costs, a fact which has also been observed in cyprinids (e.g. Ohlberger et al. 2006). However, the partial contribution of the anaerobic metabolism was not quantitatively assessed in this study. For instance, Svendsen et al. (2010) found that up to 25% of the energetic costs were associated with anaerobic metabolism in a labrifrom species. So, it is likely that total energetic costs might be increased further in Spanish toothcarp and mosquitofish after accounting for anaerobic power output. In addition, optimal swimming speeds were generally higher in *G. holbrooki* (U_{opt} mean = 6.32 $BL \cdot s^{-1}$), suggesting that this species can swim faster at optimal speeds than *A. iberus* (U_{opt} mean = 4.58 $BL \cdot s^{-1}$). Killen et al. (2016) found similar optimal swimming speeds in females of *Poecilia reticulata* (Peters, 1859), which were on average about 5 $BL \cdot s^{-1}$. Surprisingly, optimal swimming speeds were higher than critical swimming speeds in some individuals examined. This result suggests, that because of the exponential fit, the accuracy of optimal swimming speed values might be more difficult to obtain in species with low swimming capacity, since the optimal swimming speed is only affected by the speed exponent (b) from the equation (Pettersson and Hedenström, 2000).

There was no evidence of differences in standard metabolism between species. However, the standard metabolic rate varied substantially in individuals of similar size in *A. iberus*. For instance, two fishes of 70 mg had a standard metabolic rate of 0.32 and 0.01 mg O_2 h^{-1}, respectively. However, individual fish with a higher standard metabolism rate also had a higher maximal metabolic rate (0.55 and 0.18 mg $O_2 \cdot h^{-1}$, respectively), suggesting that there is a cost of maintaining the machinery that supports high aerobic capacity (Biro and Stamps, 2010). Intraspecific variation in metabolic traits of fishes within a population is a current concern (Pettersen et al. 2018), and standard metabolic rates, in particular, are thought to have important effects on some fitness correlates such as growth, reproduction and survival (Burton et al. 2011).

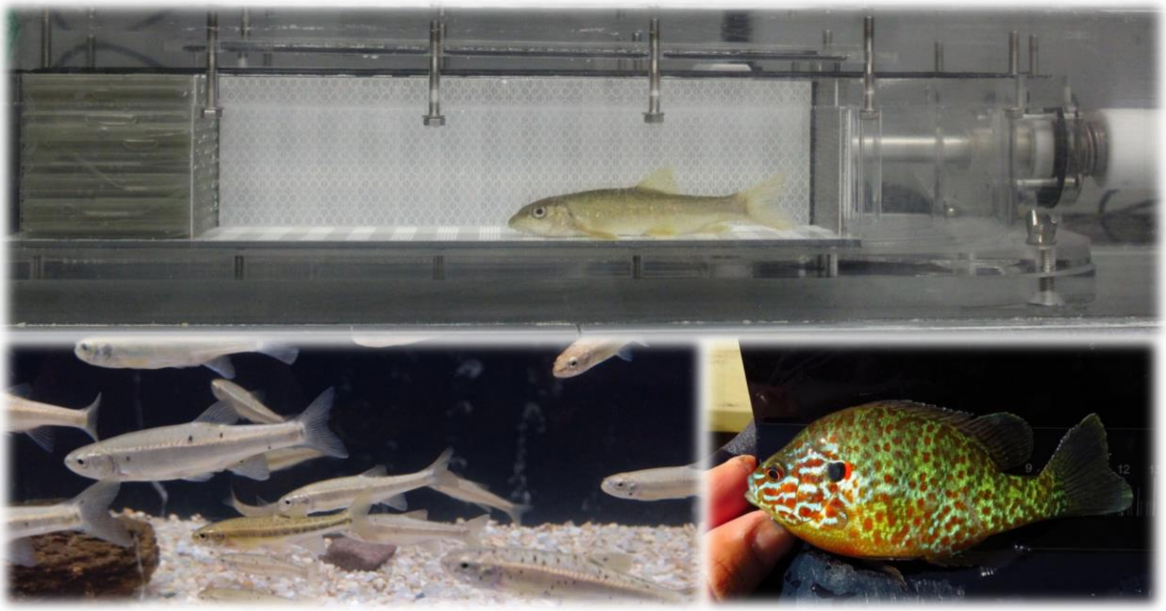
Interestingly, when comparing metabolic rates between species we found that *A. iberus* had higher maximal metabolic rate, after controlling for the effects of fish mass. This finding is, however, contrary with the notion that species reaching lower swimming speeds are expected to have more limited capacity for oxygen uptake (Tudorache et al. 2008; Norin and Clark, 2016). Even so, most of this research has been focused on comparing metabolic rates among reophilic species such as cyprinids (Yan et al. 2013) and among species with contrasting lifestyle or migratory rates (Tudorache et al. 2008; Stoffels, 2015). It is likely, therefore, that a ‘weak’ swimmer may attain higher metabolic rates at a specific speed if we compare it with other one of similar swimming capacity. Additional support for such a link might be found in the observed correlation between gait transitions (i.e. the changing use of certain body fins while swimming) with metabolic rates and locomotor performance in several fish species (Korsmeyer et al. 2002; Kendall et al. 2007; Svendsen et al. 2013). Thus, we cannot rule out that the observed differences in metabolic rates could be partly attributed to swimming gait or swimming mechanics. In addition, absolute aerobic scope was also found to be higher for *A. iberus* (AAS mean = 0.26 mg $O_2 \cdot h^{-1}$). Taken together, our results suggest that *G. holbrooki* is able to use a lower proportion of their total aerobic capacity for swimming, thereby leading to a better swimming efficiency. For instance, *A. iberus* used on average about 67% of their total aerobic scope at 2.5 $BL \cdot s^{-1}$, while *G. holbrooki* only used about 32% at the same speed. So, the invasive species had about 68% of their total aerobic scope remaining for other tasks than locomotion.

Body form is strongly linked to locomotion and swimming efficiency of species (Webb, 1984; Pettersson and Hedenström, 2000; Wainwright et al. 2002). Species with a thinner caudal peduncle can produce thrust for cruising at high speed using an axially oscillating caudal fin (Webb and Weihs, 1986; Moran, et al. 2016). In our study, both sexes of *G. holbrooki* had a narrower caudal peduncle than *A. iberus,* and even males of *G. holbrooki* had the highest caudal peduncle depth factor. Further, the significant negative correlation between the minimum cost of transport and the caudal peduncle depth factor suggests that this morphological trait seems to be especially relevant to obtain higher swimming efficiency. On the other hand, fineness ratios between 2 and 6 result in a decrease of the drag produced by the fish, with the optimum ratio for efficient swimming at about 4.5 (Webb, 1975; Langerhans and Reznick, 2010). In this study, fineness ratio was found to be higher for *G. holbrooki*, suggesting that this fish species has a more hydrodynamic shape for swimming (Ohlberger et al. 2006). In line with this hypothesis, we found a significant negative correlation of minimum cost of transport with fineness ratio, whereas there was a positive correlation with critical and optimal swimming speeds. In addition, it has been shown that fineness ratio is related to microhabitat and water flow (Scarnecchia, 1988), and therefore differences in body shape between these two species may reveal a functional trade-off between morphology and habitat preference. The more streamlined body may allow mosquitofish to swim in a variety of microhabitats (Alemadi and Jenkins, 2008), whereas the deeper body of the native toothcarp may enhance its manoeuvrability (Webb, 1994) in environments with abundant vegetation (Alcaraz and García-Berthou, 2007b).

In conclusion, we found that *G. holbrooki* performed better than *A. iberus* in the swim performance tests. Results suggest that *A. iberus* is not able to maintain steady swimming at relatively low speeds. Furthermore, energetic costs of swimming measured as cost of transport were higher in this native toothcarp, which suggest that this species requires additional energetics per unit of mass and distance compared to the invasive mosquitofish. These differences in swimming performance and efficiency can partly be explained by the habitat preferences and morphological features. This study would represent a significant contribution to address some physiological traits of these two species, and this information might be useful for ecological studies of species interactions.

CHAPTER II

Size-related effects and the influence of metabolic traits and morphology on swimming performance in fish



Rubio-Gracia F., García-Berthou E., Guasch H., Zamora L. and Vila-Gispert A. (2020). Size-related effects and the influence of metabolic traits and morphology on swimming performance in fish. *Current Zoology, 66*, 493–503

Introduction

Swimming activity and other biological processes are fuelled by energy metabolism (White et al. 2019) and, therefore, the energy budget of species plays a key role in many interactions with the physiology and ecology of organisms (Killen et al. 2010; Pang et al. 2019). The critical swimming speed test is the most common method for evaluating prolonged swimming performance (Kolok, 1999). Critical swimming speed (U_{crit}) is defined as the highest swimming speed that a fish can maintain for a time period (Brett, 1964). Although fish can rely on anaerobic metabolism while reaching U_{crit}, swimming is largely fuelled by aerobic metabolism (Videler, 1993; Hammer, 1995). Two important metabolic traits describe the bounds of a fish's respiratory capacity: maximal metabolic rate (MMR), which is defined as the maximum rate of aerobic metabolism during swimming exercise (Nelson, 2016; Norin and Clark, 2016), and standard metabolic rate (SMR), which is the baseline energy required for maintenance measured in a post-absorptive, resting state (Chabot et al. 2016).

Body size is an important factor influencing the swimming capacity and energy metabolism of fish (Beamish, 1978), and thus its effects must be accounted for before examining the variation of many traits. After accounting for body size effects, the swimming performance and metabolism of fish can vary remarkably; from inter-individual variability within species to differences among the diversity of taxonomic groups (Pettersen et al. 2018). Several studies have shown that metabolic rates such as SMR and MMR can vary two- to three-fold across individuals of the same body mass (e.g. Burton et al. 2011; Metcalfe et al. 2016), while Fisher et al. (2005) found up to 28% coefficient of variation in U_{crit} within the same species. On the other hand, because of the enormous diversity of fish species with different ecological lifestyles and behaviours, variations in metabolic rates and swimming capabilities across species are assumed to be much greater e.g., up to 40-fold difference observed by Killen et al. (2016). In general, species with a 'fast' lifestyle often have higher metabolic rates and dispersal capacities than species with a ‘slow’ lifestyle (Killen et al. 2010; Stoffels, 2015; Killen et al. 2017; Pang et al. 2019).

The association between metabolic traits, and how these are related to other aspects (e.g. morphology, behaviour and ecological correlates) have attracted interest in active areas of research in ecological and evolutionary physiology. While a mechanistic link between SMR and MMR has been observed in fish (Norin and Malte, 2012; Killen, 2014; Zhang et al. 2014), the causes of this link are still unclear. Nevertheless, there is compelling evidence that the consequences resulting from the covariation of these traits, affects biological processes such as survival, growth, predation and reproductive output (Killen et al. 2007; Burton et al. 2011; Auer et al. 2015). Additionally, MMR (and to a lesser extent SMR) has been positively associated with U_{crit} at both intra- and interspecific levels (Binning et al. 2015; Yan et al. 2013; Pang et al. 2015), thus suggesting that these traits are not entirely independent from one another. Previously, Bennett and Ruben (1979) proposed the aerobic capacity model in endotherms. Here, the authors speculated that an increased locomotor performance favours the evolution of costly physiological traits. More recently, this hypothesis has been tested in ectotherms, and suggests that selection for a high aerobic capacity to maintain intense and sustained activity is functionally supported by an increase in metabolic costs of maintenance, thereby increasing SMR (Killen et al. 2016; Pang et al. 2019). Due to their high diversity, teleost fish constitute an excellent group with which to investigate phenotypic variation in performance traits. As such, exploring the interrelationships between swimming capacity, metabolic and morphological traits may provide new insights into the mechanisms by which the locomotor performance of fish is supported.

Among the morphological traits that can affect swimming capabilities, some of the best predictors are likely to be fineness ratio (Webb, 1975; Scarnecchia, 1988; Ohlberger et al. 2006; Walker et al. 2013; Rubio-Gracia et al. 2020), caudal peduncle depth factor, propulsive ratio and muscle ratio (Fisher et al. 2000; Fisher and Hogan, 2007; Nanami, 2007). Swimming capacity is generally enhanced with a streamlined body shape, a shallow caudal region and an elevated proportion of muscle fibres (Webb, 1984; Ohlberger et al. 2006; Nanami, 2007; Domenici et al. 2008). Moreover, morphological features can influence swimming efficiency because energetic costs depend on drag forces and propulsion (Webb 1984; Videler 1993). It has been shown that streamlined bodies tend to maximize thrust and minimize drag and recoil energy losses (Webb, 1975; Ohlberger et al. 2006; Langerhans, 2009; Langerhans and Reznick, 2010). Therefore, to facilitate the understanding of swimming performance and the energetic costs of species, morphological traits must be taken into consideration.

Here, we compared eight freshwater fish species that present marked differences in body size and morphological traits. The species tested were bleak *Alburnus alburnus* (Linnaeus 1758), Catalan chub *Squalius laietanus* Doadrio, Kottelat, and de Sostoa 2007, Mediterranean barbel *Barbus meridionalis* Risso 1827, minnow *Phoxinus septimaniae* Kottelat 2007, roach *Rutilus rutilus* (Linnaeus 1758), pumpkinseed *Lepomis gibbosus* (Linnaeus 1758), Spanish toothcarp *Aphanius iberus* (Valenciennes 1846), and the eastern mosquitofish *Gambusia holbrooki* Girard 1859. The main objectives of this study were to: i) examine intra- and interspecific variation in swimming performance and energy metabolism after accounting for body size, and ii) investigate to what extent interspecific variation in swimming performance is explained by a suite of morphological and physiological traits.

Material and methods

Experimental fish and holding conditions

A total of eight species were collected between 2014 and 2017. The samples of bleak (*N* =17), Catalan chub (chub, hereafter) (*N* = 12), roach (*N* = 14) and pumpkinseed (*N* = 17) were caught from the Muga River (42°16'30.5"N 3°02'38.8"E). The sample of Mediterranean barbel

(barbel, hereafter) (N = 16) was caught from the Fluvià River (42°21’75’’N 2°57’42’’E), and the sample of minnow (N = 21) was caught from the Brugent River (42°00'09.3"N 2°36'28.4"E), a tributary of the Ter River. The different rivers were selected because the differences in the distribution ranges of the species made it difficult to collect a sufficient number of individuals from the same stream. Nonetheless, the sampling reaches were similar in terms of hydrological conditions and the specific habitat characteristics were assumed to have a negligible influence on swimming performance. In the field, fish sampling was conducted by trained personnel using electrofishing techniques. Spanish toothcarp (toothcarp, hereafter) individuals were originally collected from La Rubina salt marshes in the Alt Empordà, north-eastern Iberian Peninsula (42°15′38.7″ N, 3°8′38.9″ E) in 2012. The sample of toothcarp (N = 41) used for this study represents the fifth generation raised in captive conditions at the University of Girona. Data on swimming capacity and metabolism for mosquitofish were obtained from our previous study (Srean et al. 2017). In total, 60 mosquitofish were originally collected from the Ter Vell lagoon in L’Estartit (42°02’44”N 3°11’41’’E) in 2014 using dip nets (1 mm mesh size).

Upon arrival at the University of Girona, cyprinids and pumpkinseed were initially held in 200 l rectangular tanks, whereas toothcarp and mosquitofish were initially held in 90 l rectangular tanks. Tanks were supplied with recirculated, filtered freshwater (particle filtered and ozone sterilised) and vigorous aeration. Water changes (ca. 30% of the total volume) were conducted twice a week in each tank to assist with maintaining water quality. During the holding period, the water temperature was set to 20 ± 1°C for cyprinids, pumpkinseed and toothcarp, whereas the temperature was 25 ± 1°C for mosquitofish (Srean et al. 2017). A natural photoperiod cycle was used during the acclimation period. Fish were fed every day with frozen bloodworms (*Chironomus* sp.) using a meal size of approximately 1.5-2% of their body mass. This amount of food was enough to maintain the body condition of fish throughout the experiment, but was still below satiation levels. To avoid postprandial metabolism, fish were subjected to a fasting period of at least 24 h prior to the experiments (Secor, 2009). No mortalities occurred during the holding period and visually all fish remained in good health. Experiments were always conducted between autumn and early spring to avoid the breeding seasons of the species. In accordance with the regional legislation (Law 42/2007, Spanish Government), alien species (roach, bleak, minnow, pumpkinseed and mosquitofish) were euthanized after the experiments had taken place following a standardised protocol using concentrated MS222 in an aeriated-water holding tank. Native species, on the other hand, were released at the same place they were captured. The experiments in this study were conducted in compliance with the Autonomous Government of Catalonia (Expedient PNAE 2017PNATAAEAUT075) and the Commission of Animal Experimentation (Ref. CEA-OH/9673/1) guidelines.

Respirometer set-up

Swimming speeds and rates of oxygen consumption by eight freshwater fish species were obtained using two Blazka-style swim tunnel respirometers (Loligo® Systems, Viborg, Denmark). Both swim tunnels have a compact design that allows fish enough space for ‘free’ swimming and an adequate volume for reliable oxygen consumption measurements. Flow inside the respirometer was made rectilinear by a honeycomb plastic screen situated at the entrance of the swimming section. The continuous laminar flow was generated by a propeller connected to the motor outside of the respirometers. Swim tunnels were connected to an optical fibre instrument (Witrox 1; Loligo® Systems, Tjele, Denmark) that allowed us to record dissolved oxygen concentration $mg \cdot l^{-1}$ at 1 Hz. A temperature probe (Pt1000 temperature sensor; Witrox 1; Loligo® Systems, Tjele, Denmark) was also used for the automated compensation of oxygen data to changes in temperature and barometric pressure in real time.

Rates of oxygen consumption were measured using computerized, intermittent-flow respirometry. Swim tunnel respirometers were periodically flushed with aerated water for 2 min followed by a 1 min closed mixing period and then 20 minutes of closed respirometry.

A large swim tunnel respirometer was used for cyprinids and pumpkinseed (range of standard length, SL = 3.7−15.2 cm). The respirometer consisted of a rectangular measuring recirculation system equipped with a 5 l swimming chamber (30 cm length × 7.5 cm height × 7.5 cm width). The external water bath connected to the respirometer (25 l) was equipped with an automated Eheim pump that constantly flushed aerated water at a rate of 10 $\mathrm{l{\cdot}min^{-1}}$. Thus, we ensured the complete mixing of the respirometer water between flush cycles. We additionally connected the external water bath of the swim tunnel to a plastic supply tank containing 300 l of air-saturated freshwater. An automated Eheim pump continuously provided freshwater from the supply tank to the external water bath, and then water was recirculated again through a decantation system. The supply tank was equipped with an automated liquid cooler (85 W, 972.46 BTU/h, J.P Selecta®) to maintain the temperature at 20 °C.

A small swim tunnel respirometer was used for toothcarp and mosquitofish (range SL = 1.2−3.6 cm). The respirometer consisted of a 170 ml tubular swimming chamber (100 mm length × 26.4 mm internal diameter) immersed in an external water bath containing 25 l of clean, aerated water. The respirometer was darkened on the outside with black plastic to prevent external disturbances. An automated Eheim pump replaced freshwater inside the chamber at a rate of 5 $\mathrm{l{\cdot}min^{-1}}$. For mosquitofish, the water temperature was directly controlled at 25 ± 1°C using an Eheim heater placed in the external water bath.

Determination of swimming performance and metabolic traits

Essentially, we followed the same methodology and procedures used in our previous work with mosquitofish (Srean et al. 2017). After at least 2 weeks of being held under experimental conditions, fish were then individually transferred into the swim tunnel respirometer. Any effects from the stress of being handled were potentially minimised by transferring fish into the swimming flume without exposing them to air and providing them with a 1-h acclimation period at a speed of 0.5 $\mathrm{BL \cdot s^{-1}}$ (body length, taken as the standard length of the fish). A critical swimming speed (U_{crit}) test was performed with step-wise increases in flow speed of approximately 1 $\mathrm{BL \cdot s^{-1}}$ with a time interval of 20 min until the fish fatigued. Fatigue was defined as having occurred when the fish could no longer swim against the current continuously (Farrell, 2008). We calculated U_{crit} according to Beamish (1978):

$$U_{crit} = U_f \ + U_i T_f T_i^{-1},$$

where U_f is the highest velocity maintained for the entire swimming period, U_i is the speed increment ($\mathrm{cm \cdot s^{-1}}$), T_f is the time elapsed at fatigue speed and T_i is the set interval time (min). When the calculated cross-section of the fish was more that 10% of the swimming tunnel cross-section, swimming speed was corrected for the solid blocking effect (Bell and Terhune, 1970). The relative U_{crit} was calculated by dividing U_{crit} values by standard length.

Measurements of oxygen consumption of individual fish were recorded during swimming. Linear regressions were fitted from the decline in oxygen concentration in the respirometers over time between flush cycles. The 20:2 min measurement: flush cycle produced clear slopes of oxygen concentration during the measurement period. Regression slopes were then used to calculate oxygen consumption rates:

$$\dot{M}_{\mathrm{O_2}} = -(\Delta Of - \Delta Ob) \times V \times 60,$$

where ΔOf and ΔOb are the slopes of the regression (mg $\mathrm{O_2}$ $\mathrm{l^{-1} \cdot min^{-1}}$) due to fish respiration and microbial respiration, respectively, V is the volume of the respirometer (after subtracting the fish volume) and 60 was used to change units to mg $\mathrm{O_2 \cdot h^{-1}}$. The highest value of $\dot{M}_{\mathrm{O_2}}$ during the swimming trial was defined as the maximal metabolic rate (MMR) (Nelson, 2016;

Norin and Clark, 2016). At the end of each swimming trial, the respirometer was left without fish for 10 min to calculate the background respiration associated to microbial activity.

The exponential function generally resulted in higher regression coefficients than the power function did and was therefore used as the model to describe the relationship between $\dot{M}_{\mathrm{O}_2}$ (log-transformed) and swimming speed (U) (Brett, 1964; Webb, 1975; Beamish, 1978; Tudorache et al. 2008):

$$\dot{M}_{\mathrm{O}_2} = \mathrm{SMR} \times e^{bU},$$

where SMR is the estimated standard metabolic rate at zero swimming speed and b an estimated constant that can be used as an index of swimming efficiency; that is, the higher the value of b, the more marked the increase in the swimming $\dot{M}_{\mathrm{O}_2}$ with increased swimming speed. The mass-specific cost of transport (MCOT) was calculated by dividing the obtained $\dot{M}_{\mathrm{O}_2}$ values by the corresponding speed and body mass. MCOT values were then converted into energy units using an oxycaloric value of 14.1 $\mathrm{J \cdot mgO_2^{-1}}$ (Hepher, 1988).

Fish morphology

Following the swimming trials, individuals were weighed to the nearest 0.1 mg and some morphological measurements were obtained from digital images using tpsDig2 software (Rohlf, 2015). These measurements included lengths (standard length and total length), depths (maximum body depth and least depth of caudal peduncle) and areas (body, muscle and propulsion areas). Some morphometric ratios were then calculated following Fisher and Hogan (2007): fineness ratio (ratio between standard length and maximum body depth), caudal peduncle depth factor (ratio between caudal peduncle depth and maximum body depth), propulsive ratio (ratio between propulsive area and body area) and muscle ratio (ratio between muscle area and body area).

Data analysis

Statistical analyses were performed in the R software environment (R Development Core Team 2018). The individual effects of body mass and total length on U_{crit}, MMR and SMR were analysed by linear regression. Following Srean et al. (2017), we performed multiple linear regressions using total length, MMR or SMR as covariates, once body mass has been accounted for, thus allowing us to assess potential independent effects of body mass and an additional covariate (independent variables). When the two regression coefficients were significant, we repeated the same model separately by sexes to account for sex-dependent effects. The suitability of different models was assessed by the significance of regression coefficients and the likelihood ratio tests. The effects of body size on swimming $\dot{M}_{\mathrm{O}_2}$ were evaluated by linearizing the exponential function (i.e. $\dot{M}_{\mathrm{O}_2}$ was $\log_{10}$-transformed). Analysis of covariance (ANCOVA) was applied to compare $\dot{M}_{\mathrm{O}_2}$ across different body mass ranges within species using swimming speed as the covariate. ANCOVA was also applied to test for differences in U_{crit}, MMR and SMR among fish species using body mass as the covariate. Regression slopes were only compared between the five cyprinids and pumpkinseed because of the enormous difference in size between these species and the other two remaining species (toothcarp and mosquitofish). In addition, ANCOVA was not applied to compare toothcarp and mosquitofish due to differences in origin (i.e. captive-bred stock and wild population) and experimental temperature. Tukey's multiple comparison test was applied to elucidate differences in covariate adjusted means among species. Metabolic rates were standardised to the mean of body mass according to $\dot{M}_{\mathrm{O}_{2(4.65\ \mathrm{g})}} = \dot{M}_{\mathrm{O}_{2\mathrm{observed}}} \times (4.65/M)^b$, where b is a common scaling coefficient of 0.75 used for all individuals (e.g. Reidy et al. 2000). Interrelationships between swimming capacity, metabolic traits and morphometric ratios were evaluated using linear regression analysis and Pearson-product moment correlations. Individual fractions of variation explained by 'species' and morphometric ratios (all of them pooled) were tested using variation partitioning (the ‘varpart’ function of the vegan package in R). Significance of testable fractions was determined using 999 permutation tests with the ‘anova.cca’ function (Oksanen et al. 2017).

Results

Intraspecific variation

Body mass was largely the best predictor of U_{crit}, SMR and MMR (R^2 values were always higher for body mass than for total length). The inclusion of a second predictor such as total length or MMR in addition to body mass markedly increased the explained variation of U_{crit} (range = 6.7−480% increase), whereas the inclusion of SMR improved the explained variation of MMR (range = 12−135% increase) in several species (Table 1). Additionally, we found that body mass and total length were both significant for U_{crit} in minnow, and body mass and MMR were both significant for U_{crit} in toothcarp and mosquitofish (Table 1). U_{crit} was only affected independently by the two predictors in minnow and mosquitofish. This result therefore indicates that, for instance, longer minnow individuals of similar body mass had lower U_{crit}. When testing this model separately for each sex, we found that the two predictors were only significant in minnow males ($\log_{10} U_{\mathrm{crit}} = 3.43 + 1.38 \log_{10} M – 2.82 \log_{10} \mathrm{TL}$; $R^2_{\mathrm{adj}} = 0.80$). Moreover, we found that body mass and SMR were both significant for MMR in mosquitofish (Table 1). When testing this model separately for each sex, we found that the two predictors were only significant in mosquitofish females ($\log_{10} \mathrm{MMR} = 0.13 + 0.40 \log_{10} M + 0.26 \log_{10} \mathrm{SMR}$; $R^2_{\mathrm{adj}} = 0.76$), indicating that individuals of the same body mass with higher SMR also had higher MMR.

Oxygen consumption was highly correlated with swimming speed, with the speed exponent *b* ranging from 0.01 to 0.09 (Table S1). The value of the speed exponent generally tended to decrease with increasing body mass, which indicates that heavier fish can optimally swim faster. Importantly, species showed high intraspecific variation in $\dot{M}_{O_2}$ across mass-range groups (Figure 1). ANCOVA showed significant effects of body mass on $\dot{M}_{O_2}$ in six fish species, with the exception of minnow ($F_{1,\,160}$ = 0.03, P = 0.97) and toothcarp ($F_{1,\,213}$ = 1.48, P = 0.22).

T A B L E 1 Simple and multiple linear regression analyses of the swimming performance and metabolic traits in 8 freshwater fish species

	Dependent variable	Independent variables		a	b_1	b_2	R^2_{adj}
Bleak	U_{crit}	M		1.30***	0.66*		0.42^a
	U_{crit}	M	TL	0.54	0.36	1.0	0.44^a
	U_{crit}	M	MMR	1.41***	0.36	0.24	0.47^a
	MMR	M		-0.47	1.27***		0.51^a
	MMR	M	TL	0.07	1.48*	-0.70	0.49^a
	MMR	M	SMR	-0.61	1.39**	-0.14	0.49^a
Catalan chub	U_{crit}	M		1.46***	0.36***		0.85^a
	U_{crit}	M	TL	1.17***	0.23	0.4	0.85^a
	U_{crit}	M	MMR	1.42***	0.31	0.11	0.84^a
	MMR	M		0.39**	0.41**		0.60^a
	MMR	M	TL	0.5	0.46	-0.15	0.56^a
	MMR	M	SMR	0.41**	0.31*	0.3	0.64^a
Mediterranean barbel	U_{crit}	M		1.65***	0.13**		0.44^a
	U_{crit}	M	TL	0.7	-0.26	1.30*	0.61^b
	U_{crit}	M	MMR	1.65***	0.13	-0.01	0.40^a
	MMR	M		0.29**	0.63***		0.78^a
	MMR	M	TL	-0.64	0.26	1.27	0.78^a
	MMR	M	SMR	0.37*	0.51*	0.15	0.77^a
Minnow	U_{crit}	M		1.30***	0.83***		0.75^a
	U_{crit}	M	TL	3.28***	1.26***	-2.56*	0.80^b
	U_{crit}	M	MMR	1.30***	0.79***	0.04	0.74^a
	MMR	M		-0.05	0.92***		0.52^a
	MMR	M	TL	-1.82	0.54	2.29	0.52^a
	MMR	M	SMR	-0.04	0.80**	0.15	0.51^a
Roach	U_{crit}	M		1.38***	0.52**		0.50^a

	U_{crit}	M		1.38***	0.52**		0.50^a
	U_{crit}	M	TL	3.57	1.54	-3.11	0.50^a
	U_{crit}	M	MMR	1.51***	-0.01	0.44**	0.73^b
	MMR	M		-0.29	1.22***		0.68^a
	MMR	M	TL	-2.96	-0.02	3.79	0.67^a
	MMR	M	SMR	-0.44	1.40**	-0.13	0.67^a
Pumpkinseed	U_{crit}	M		0.76	0.46		0.05^a
	U_{crit}	M	TL	4.26	2.14	-5.54	0.03^a
	U_{crit}	M	MMR	1.41**	-0.33	0.47*	0.29^b
	MMR	M		-1.38**	1.69***		0.50^a
	MMR	M	TL	-0.81	1.96	-0.9	0.47^a
	MMR	M	SMR	-1.79*	2.09**	-0.21	0.49^a
Spanish toothcarp	U_{crit}	M		1.22***	0.40**		0.22^a
	U_{crit}	M	TL	-0.65	-0.51	3.03	0.27^a
	U_{crit}	M	MMR	1.39***	0.34**	0.29***	0.43^b
	MMR	M		-0.43***	0.43*		0.11^a
	MMR	M	TL	-3.48*	-1.10	4.88*	0.17^b
	MMR	M	SMR	-0.08	-0.18	0.42***	0.40^c
Mosquitofish	U_{crit}	M		1.28***	0.19**		0.15^a
	U_{crit}	M	TL	1.25*	0.18	0.03	0.13^a
	U_{crit}	M	MMR	1.48***	-0.20**	0.71***	0.63^b
	MMR	M		-0.29***	0.55***		0.57^a
	MMR	M	TL	0.45	0.91**	-1.09	0.58^a
	MMR	M	SMR	-0.14*	0.40***	0.22**	0.64^b

The 2 regression coefficients were not significant in models with SMR as the dependent variable, hence linear regression functions have been omitted from the table. The different lowercase letters show significant differences between models (likelihood ratio tests, $P < 0.05$). M, body mass; MMR, maximal metabolic rate; SMR, standard metabolic rate; TL, total length; and U_{crit}, critical swimming speed. All variables were $\log_{10}$-transformed (*$P \leq 0.05$, **$P \leq 0.01$, and ***$P \leq 0.001$)

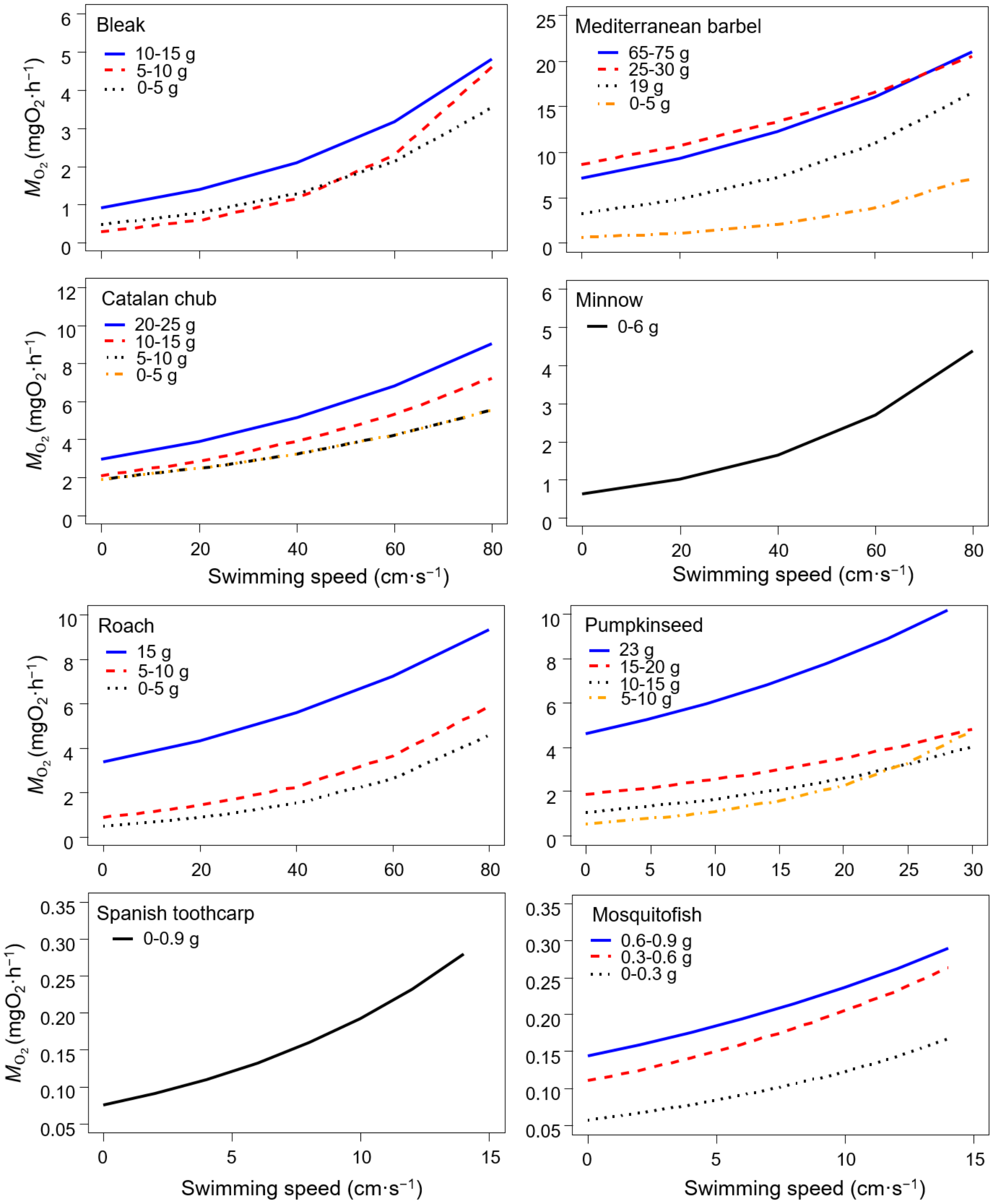
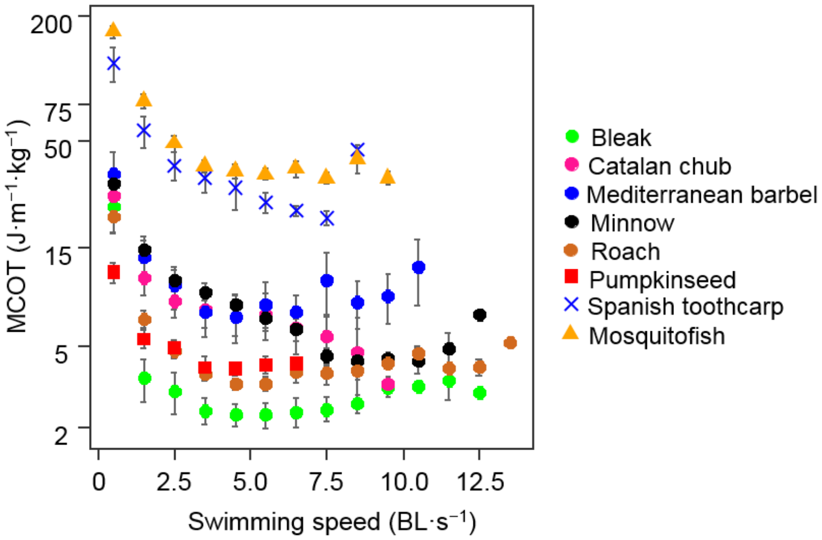


FIGURE 1 Metabolic rates ($\dot{M}_{O_2}$) as a function of swimming speed measured in 8 freshwater fish species. Within each species, fish were grouped into different mass-range groups according to their respective body mass: ranges of 5 g for bleak, Catalan chub, Mediterranean barbel, roach, and pumpkinseed; 2 g for minnow, and 0.3 g for Spanish toothcarp and mosquitofish. An exponential function is shown for each range of body mass within species when there were significant differences. For $\log_{10}$-transformed regression equations, see Supplementary Table S1

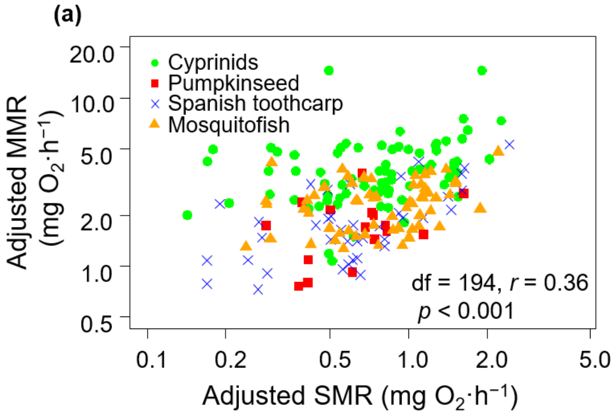
Interspecific variation

ANCOVA showed a significant mass × species interaction for U_{crit} for barbel ($P = 0.003$) but not for the rest of species, which had similar slopes (Table 1) (Figure S1a). After accounting for body mass effects, minnow and bleak had higher U_{crit} than chub (Tukey test, $P = 0.02$ and $P = 0.04$, respectively), and cyprinids had significantly higher U_{crit} than pumpkinseed (ANCOVA, $F_{4,\,75}$=85.29, $P < 0.001$). In addition, bleak had the lowest SMR ($F_{5,\,90}$=11.16, $P <$ 0.001) (Figure S1b). MMR values were generally higher in barbel and chub, although ANCOVA showed a significant mass × species interaction for these species (barbel: $P = 0.03$; chub: $P = 0.01$) (Figure S1c). Minnow had higher MMR than bleak (Tukey test, $P < 0.002$), and cyprinids had higher MMR than pumpkinseed ($F_{3,\,64} = 18.13$, $P < 0.01$). Toothcarp and mosquitofish attained the highest MCOT at a comparable workload, whereas bleak showed the lowest MCOT (Figure 2). Interestingly, pumpkinseed attained lower MCOT than some cyprinids at a comparable workload.



F I G U R E 2 Mass-specific cost of transport (MCOT) as a function of swimming speed measured in 8 freshwater fish species. MCOT was negatively related with swimming speed ($P < 0.001$). Metabolic rates (in $J \cdot m^{-1} \cdot kg^{-1}$) are shown as means and error bars at each swimming speed presented as body length per second ($BL \cdot s^{-1}$). Note that data represent oxygen uptake at a comparable workload, but not necessarily at a comparable speed. Y-axis is on a $\log_{10}$ scale. Cyprinid species are represented by circles

We found that SMR and MMR were positively correlated (Figure 3a), following the general relation $\log_{10}$ MMR = 0.45 + 0.34 $\log_{10}$ SMR ($P < 0.001$, $R^2 = 0.14$) after adjusting for body mass. Further, U_{crit} and MMR were also positively correlated (Figure 3b), following the general relation $\log_{10}$ MMR = –0.17 + 0.78 $\log_{10}$ U_{crit} ($P < 0.001$, $R^2 = 0.40$), after adjusting for body mass. Linear models without mosquitofish (the only species measured at 25 °C) showed similar explained variation as the models including all the species ($R^2 = 0.15$ and 0.40). The adjusted estimates of SMR and MMR and the relative U_{crit} showed a 15, 19-, and 7-fold interspecific difference between minimum and maximum values, respectively, which highlights high variation in metabolic traits and swimming capabilities across fish species. The mean values of the morphometric ratios for each species are presented in Table 2. The linear relationships of the adjusted estimates of SMR and MMR and the relative U_{crit} with morphometric ratios are presented in Table 3. The adjusted MMR was significantly correlated with the muscle and fineness ratios (Figures 4a-b). 'Species' (18%; $F_{7,\,148} = 8.22$, $P = 0.001$), and morphometric ratios and 'species' together (23%; $F_{4,\,155} = 12.23$, $P = 0.001$) significantly contributed to explaining the variation in MMR (variation partitioning). The relative U_{crit} was also significantly correlated with the muscle and fineness ratios (Figures 4c-d). 'Species' (9%; $F_{7,\,150} = 5.72$, $P = 0.001$), morphometric ratios (2%; $F_{4,\,150} = 2.38$, $P = 0.048$), and both predictors together (47%; $F_{4,\,157} = 38.49$, $P = 0.001$) were found to be significant. The interrelationships between MMR, U_{crit} and morphometric ratios reflected a gradient of performance among species described by pumpkinseed < toothcarp < mosquitofish < cyprinids (pooled). In contrast, the variation in SMR was only explained by the contribution of 'species' (18%; $F_{7,\,149} = 6.18$, $P = 0.001$), with morphometric ratios having negligible effects.



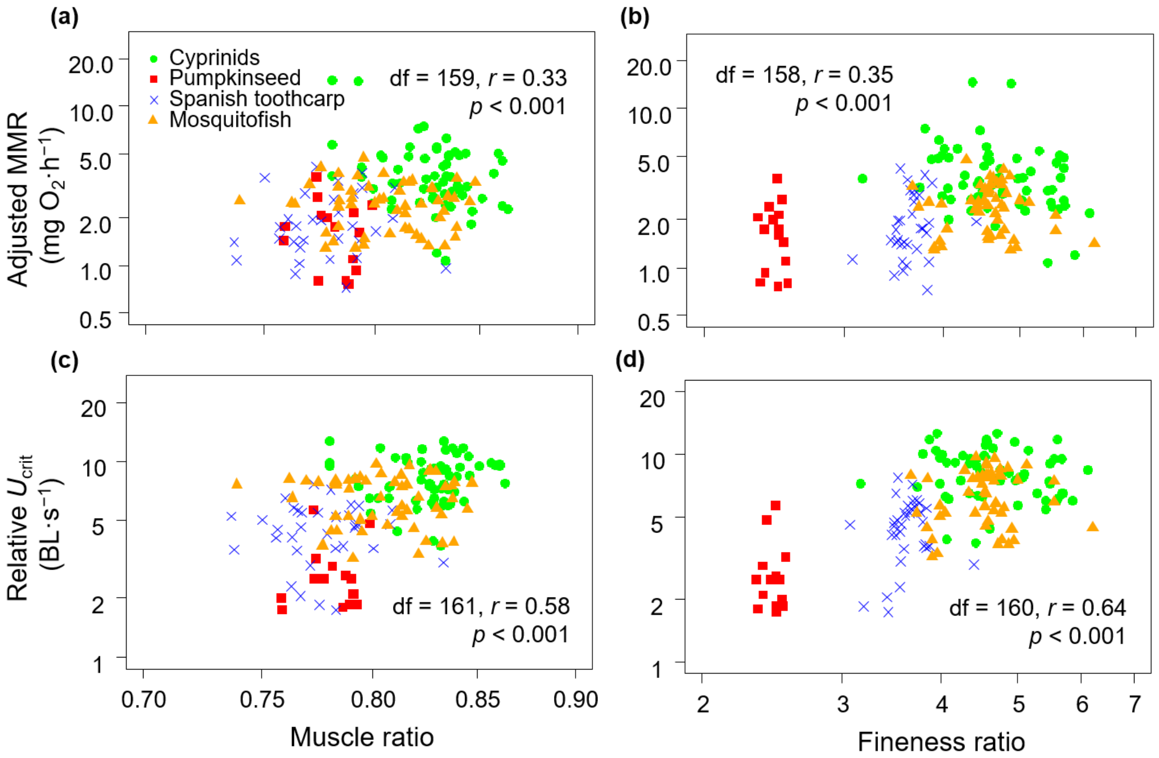
F I G U R E 3 Relationships of adjusted standard metabolic rate (SMR) (a) and relative critical swimming speed (U_{crit}) (b) with adjusted maximal metabolic rate (MMR). Metabolic rates shown were standardized to the mean of body mass (4.65 g). For clarity, the 5 cyprinid species were pooled and shown as cyprinids. Relationships were significantly positive in both cases. Both axes are on a $\log_{10}$ scale

TABLE 2 Means ± standard deviations of various morphometric ratios measured in the eight freshwater fish species studied

	N	Fineness ratio	Muscle ratio	Propulsive ratio	Caudal peduncle depth factor
Bleak	17	5.30 ± 0.55	0.84 ± 0.01	1.13 ± 0.01	0.46 ± 0.05
Catalan chub	7	4.58 ± 0.25	0.81 ± 0.02	0.99 ± 0.02	0.46 ± 0.03
Mediterranean barbel	6	4.43 ± 0.38	0.79 ± 0.02	1.03 ± 0.02	0.49 ± 0.02
Minnow	19	4.89 ± 0.45	0.83 ± 0.02	1.12 ± 0.02	0.47 ± 0.03
Roach	12	3.92 ± 0.26	0.83 ± 0.01	1.09 ± 0.01	0.37 ± 0.02
Pumpkinseed	15	2.46 ± 0.07	0.78 ± 0.01	1.23 ± 0.01	0.32 ± 0.01
Spanish toothcarp	34	3.63 ± 0.22	0.78 ± 0.02	0.96 ± 0.02	0.60 ± 0.04
Mosquitofish	52	4.56 ± 0.46	0.81 ± 0.02	1.05 ± 0.02	0.64 ± 0.06

TABLE 3 Linear relationships of relative critical swimming speed (U_{crit}), adjusted standard metabolic rate (SMR), and adjusted maximal metabolic rate (MMR) (metabolic rates were standardized to the mean of body mass, 4.65 g) with various morphometric ratios

	U_{crit} (BL·s^{-1})					SMR (mg O$_2$·h^{-1})					MMR (mg O$_2$·h^{-1})				
	Estimate ± SE	df	*F*	*P*	R^2	Estimate ± SE	df	*F*	*P*	R^2	Estimate ± SE	df	*F*	*P*	R^2
Fineness ratio	0.303 ± 0.029	160	111.4	***	0.41	0.022 ± 0.031	159	0.51	0.48	0.003	0.149 ± 0.031	158	22.62	***	0.13
Muscle ratio	0.036 ± 0.005	161	47.11	***	0.23	-0.003 ± 0.005	160	0.39	0.54	0.002	0.022 ± 0.005	159	18.9	***	0.11
Propulsive ratio	-0.014 ± 0.014	161	1.07	0.3	0.01	-0.014 ± 0.011	160	1.4	0.23	0.009	0.003 ± 0.012	159	0.04	0.84	<0.001
Caudal peduncle depth factor	0.072 ± 0.040	160	3.21	0.07	0.02	0.039 ± 0.033	160	1.34	0.25	0.008	-0.048 ± 0.036	159	1.73	0.19	0.01



F I G U R E 4 Interspecific variation in adjusted maximal metabolic rate (MMR) and relative critical swimming speed (U_{crit}) across 8 freshwater fish species in relation to 2 morphometric ratios. Adjusted estimates of MMR and U_{crit} plotted against muscle ratio (a, c) and fineness ratio (b, d). For clarity, the five cyprinid species were pooled and shown as cyprinids. All relationships were significantly positive (Table 3), indicating that these two morphological traits influenced swimming capacity and aerobic metabolism. Both axes are on a $\log_{10}$ scale

Discussion

Although body mass and total length are both indicators of size in fish (Beamish, 1978), we found stronger positive relationships between body mass and both U_{crit} and metabolic traits. The effects of body mass on metabolic traits are not surprising as energy turnover varies according to body constituents (Killen et al. 2010; White et al. 2019). Nevertheless, some previous studies have used total length for predicting U_{crit} in fish (Ojanguren and Braña, 2003; Hogan et al. 2007; Mateus et al. 2008). It appears, however, that body mass may better predict fish swimming performance because it is directly related to body volume and, consequently, to swimming thrust (Ohlberger et al. 2005; Srean et al. 2017). We also observed that body mass influenced the swimming efficiency of most of the species studied, indicating that heavier fish are able to optimise energetic costs per unit of mass and distance due to a more favourable ratio of body volume to body surface in larger fish (Webb, 1975; Beamish, 1978; Videler, 1993). However, toothcarp and minnow did not show differences in metabolic rates across individuals of different body mass. For minnow, this fact could be related to the restricted size range of individuals analysed, as this species is notably smaller than the rest of cyprinids studied, and it would have also been necessary to use minnow juveniles in a smaller swim tunnel. In contrast, we used a relatively similar body size range for toothcarp and mosquitofish and, surprisingly, body mass markedly influenced the metabolic rates only in mosquitofish. This latter species also showed higher swimming efficiency (i.e. lower speed exponents) than toothcarp, indicating that these two species might differ in the way energy metabolism is used while swimming. In agreement with this, Rubio-Gracia et al. (2020) tested the two species at 25 °C and found that mosquitofish can optimally swim without excessively increasing energetic costs over a wider range of swimming speeds. In short, this study shows the importance body mass has on influencing swimming performance and energy use.

In this study, U_{crit} was affected independently by body mass and total length in minnow and by body mass and MMR in mosquitofish. These results, along with other studies evaluating swimming performance in certain fish species (e.g. Ohlberger et al. 2005; Srean et al. 2017), are consistent with the idea that, after accounting for body size, the sign of the relationships between swimming capacity and other traits may not always be positive. In relation to mosquitofish, Srean et al. (2017) pointed out that negative associations between U_{crit} and MMR can be related to the fact that females generally display more variability in body size and shape than males do. However, this is not likely to be the case for toothcarp, as females are generally larger than males. Instead, both sexes seem to have similar morphological traits related to swimming performance (Rubio-Gracia et al. 2020), and this would most likely explain the positive associations observed between traits. In short, this study opens a path to further investigate the sign and causes of the relationships between swimming capacity and metabolic traits in fish species, and the consequences trait covariation has on ecological interactions.

We found considerably high variation in SMR, even after standardizing to a common body mass. This could likely be related to the short acclimation period (1 h) used for the fish before starting the U_{crit} protocol. In general, several hours are needed to obtain the best accuracy of SMR estimates, although that said, the duration of acclimation period depends on the species (Chabot et al. 2016). Thus, taking this into consideration, SMR estimates (obtained from the $\dot{M}_{O_2}$-swimming curve) of species might have been somewhat overestimated. By contrast, estimates of U_{crit} were not likely affected by acclimation period since previous studies have shown that the U_{crit} protocol is relatively robust to variation in methodological procedures (Hogan et al. 2007). Another important aspect is that mosquitofish swam at 25°C in the swim tunnel, which represents an experimental temperature five degrees warmer with respect to the other species. Because of this, we avoided the direct comparison of mosquitofish with the rest of the species, since a potential bias of metabolic rates and swimming performance would be expected due to the strong dependency of oxygen uptake with temperature in ectotherms (Fry and Hart, 1948; Ohlberger et al. 2007). Nevertheless, we found that the explained variation of MMR by SMR was similar when removing mosquitofish from predictive models. This may be explained by the fact that mosquitofish is a highly eurythermal fish (Otto, 2006) and metabolic rates are supposed to raise slowly as temperature increases (Schulte, 2015), while abrupt changes in swimming performance seems to occur at more extreme temperatures (Wilson, 2005). Therefore, this finding suggests that the strength of the relationship between metabolic traits may not be affected by small changes in temperature.

At the interspecific level, minnow and bleak displayed higher U_{crit} than other cyprinids, and all cyprinids displayed higher U_{crit} and MMR than pumpkinseed for a given body mass. Thus, an improved swimming capacity was associated with higher metabolic capacity, as noted in previous studies (Yan et al. 2013). Differences in swimming capacity among species can be partly explained by the significant positive relationships between U_{crit} and the fineness and muscle ratios of species. Effects of these morphometric ratios on swimming capabilities have been previously reported in several fish species (Fisher et al. 2000; Ohlberger et al. 2006; Nanami, 2007; Rubio-Gracia et al. 2020). Nanami (2007) pointed out that species with an elevated proportion of muscle fibres and slender body shape can generate more forward thrust and reach higher speeds. In addition to U_{crit}, we found that MMR was also positively correlated with fineness and muscle ratios, whereas the effects of these two morphometric ratios were negligible on SMR. Even though we are not certain whether the high variation observed in SMR may have counteracted the strength of the relationship between SMR and morphological traits, our results are compatible with much broader scale studies that explored the interrelationships of these traits in teleost fish. For instance, Killen et al. (2016) examined the interrelationships between metabolic and morphological traits among 92 fish species and found that some morphological traits (gill surface area, muscle protein and caudal fin aspect ratio) had greater effects on MMR than on SMR. Interspecific variation may also be explained by the differences in ecological lifestyle among species (Killen et al. 2010; Stoffels, 2015; Killen et al. 2017), since cyprinids are likely to be more rheophilic species than the other three species studied (Cano-Barbacil et al. 2020b). However, differences in metabolic and dispersal rates among the species would likely be greater if we had studied a species with a clear fast-flow lifestyle. Species with benthic and benthopelagic behaviour can have relatively similar metabolic traits, unlike species with pelagic behaviour which clearly attain higher metabolic traits (e.g. Stoffels, 2015). Taken together, this study shows that interspecific variation in U_{crit} can be partly explained by the variation in the interrelated traits of MMR, fineness and muscle ratios.

Pumpkinseed, which is characterised by a very deep and laterally compressed body, attained lower mass-specific cost of transport than some cyprinids, which tend to have more fusiform or streamlined bodies, did. This finding may be explained by the relatively similar levels of SMR among the species. For instance, Pettersson and Brönmark (1999) found that deep-bodied fish did not experience any higher costs of transport than shallow-bodied fish because of their lower SMR, thereby compensating potential hydrodynamic disadvantages associated with high-drag morphs. Nevertheless, the cost of transport increases sharply in deep-bodied species when swimming speed is increased (Pettersson and Hedeström, 2000), and therefore cyprinids are supposed to swim more efficiently at a wider range of speeds. Ultimately, other unexplored factors such as swimming mode and mechanics might also have influenced the swimming efficiency of the species (Fulton, 2007). Species from the genus *Lepomis* often use median and paired-fin swimming (labriform swimming) (Jones et al. 2007), and previous studies have found that this type of swimming is energetically less costly than undulatory swimming (Korsmeyer et al. 2002), which is often employed by cyprinids at intermediate and high speeds.

Therefore, this finding provides evidence supporting the idea that fish species with high-drag morphs may show cost reduction strategies to compensate for hydrodynamic disadvantages.

CHAPTER III

Combined effects of hydrologic alteration and cyprinid fish in mediating biogeochemical processes in a Mediterranean stream

Rubio-Gracia F., Almeida D., Bonet B., Casals F., Espinosa C., Flecker A. S., García-Berthou E., Martí E., Tuulaikhuu B-A., Vila-Gispert A., Zamora L. and Guasch H. (2017). Combined effects of hydrologic alteration and cyprinid fish in mediating biogeochemical processes in a Mediterranean stream. *Science of the Total Environment*, *601–602*, 1217–1225

Introduction

Rivers are affected by multiple environmental stressors including, among others, the frequency and intensity of flooding or drying episodes over an annual cycle. In Mediterranean streams, water abstraction by irrigation weirs is one of the most frequent anthropogenic alterations that causes strong changes in hydrologic regimes over the year. Irrigation channels connecting reservoirs to irrigated areas often maintain regular discharge during some periods, while in other instances weirs can extend the intensity of water intermittency, since maximum water demand coincides with the driest period in summer (Rosenberg et al. 2000). Weirs not only influence the quantity of water but also biotic communities such as macroinvertebrates and fish (Mueller et al. 2011). In addition, streams draining catchments subjected to land use changes derived from human activities, especially agriculture, display higher nutrient concentrations (Bennett et al. 2001; Boyer et al. 2002), which can further affect in-stream benthic communities and biogeochemical processes.

Regarding biogeochemical processes and their interactions with biotic communities, a reduction in flow leads in many cases to higher nutrient concentrations, affecting not only algal growth but also macroinvertebrate density (Robinson et al. 2014). Macroinvertebrates and fish can also modify nutrient concentrations affecting algal primary productivity (Vanni, 2002; Bracken, 2004; McIntyre et al. 2008; Capps and Flecker, 2013). However, this influence of consumer nutrient recycling may not occur in eutrophic environments where nutrient availability may not act as a limiting factor. Under these conditions, the contribution of consumers in supplying nutrients may become undetectable due to nutrient saturation (Spooner et al. 2013).

It is important to note that periphyton and invertebrates may quickly recover after extreme flow events due to their short life-cycles and high dispersal abilities (Blueweiss et al. 1978; Bonada et al. 2007). In contrast, we expect a stronger effect of hydrologic alteration on fish populations than on smaller-size organisms with higher colonization rates (Allan and Flecker, 1993; Matthews and Marsh-Matthews, 2003). During dry periods, fish populations are confined to flow refuges, increasing their density and potentially the intensity of predator-prey interactions and competition for space (Spranza and Stanley, 2000; Lake, 2003; Magoulick and Kobza, 2003). However, alterations of the flow regime may affect maturation and spawning, thereby leading to a further reduction of density (lower recruitment) and species richness after rewetting (Humphries et al. 2008; Merciai et al. 2017).

Although there has long been interest in understanding the effects of flow alteration on stream communities and biogeochemical processes (e.g. Timoner et al. 2014), the link between community changes and biogeochemistry under different flow regimes is not clear cut.

As for periphyton, complex microbial assemblages, which include bacteria, algae, fungi and protozoa within a matrix of extracellular polymeric substances (Romaní et al. 2012), play a fundamental role in in-stream biogeochemical cycles through influencing nutrient uptake, transfer of nutrients to higher trophic levels, and remineralization (von Schiller et al. 2007). Variation in dissolved inorganic nitrogen (N) uptake among streams can depend on the specific rates associated with different microbial processes including assimilatory uptake, nitrification, denitrification, adsorption-desorption (Peterson et al. 2001; Mulholland et al. 2008; Merbt et al. 2011; Ribot et al. 2017). In contrast, uptake of dissolved inorganic phosphorus (P) is mainly driven by assimilatory uptake and chemical co-precipitation (Dodds, 2003). Since these biogeochemical processes associated with periphyton are influenced by prevailing physical and chemical conditions (Sabater et al. 2002b), it is likely that hydrologic alteration, and more specifically flow intermittence, can affect stream nutrient dynamics. However, several investigations indicate that after a drying period, rewetting leads to fast colonization and recovery of periphyton (e.g. Timoner et al. 2014). The question here is whether periphyton communities from intermittent streams respond similarly to an additional alteration (i.e. the presence or absence of fish) as communities growing under permanent flow conditions.

The main goal of this study was to compare the structural and functional response of two stream reaches differing in terms of hydrologic alteration, to additional alterations associated with the loss or increased density of fish. These scenarios are likely to co-occur since, as mentioned above, hydrologic alteration may directly affect the density, survival and further colonization of fish. We performed a field mesocosm experiment in two reaches of a eutrophic stream using cages where we aimed to modify the density of the Mediterranean barbel *Barbus meridionalis* (‘barbel’, hereafter). The Mediterranean barbel is a small-bodied cyprinid fish, endemic to Mediterranean streams of the NE Iberian Peninsula and southern France. It has been classified as a benthic insectivore that feeds primarily on chironomid larvae and Ephemeroptera nymphs, although detritus is also an important food source (Mas-Martí et al. 2010). At each reach and for each fish density treatment, we estimated biomass, elemental content and nutrient uptake (i.e. of ammonia and phosphate) as response variables for periphyton, and biomass and density as response variables for macroinvertebrate community structure. Our main hypothesis was that the effects on the structure and function of fluvial communities from hydrologic alterations that cause long periods of dryness, will persist after rewetting, thereby influencing their response to the presence or absence of fish.

Material and methods

Ethics statements

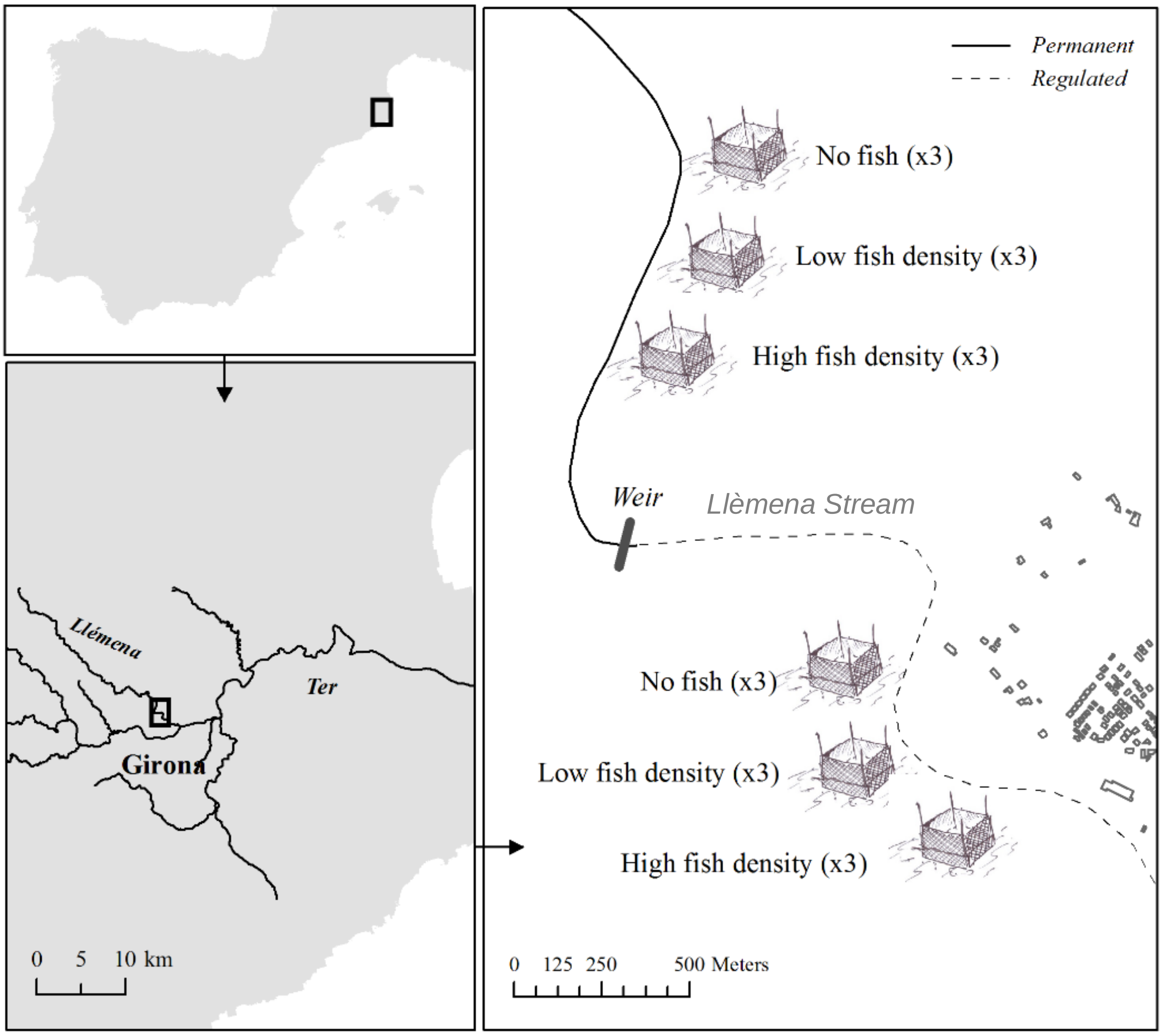
This study was authorized by the Autonomous Government of Catalonia (Generalitat de Catalunya) and we received the permit required to carry out the reported fieldwork. Animal manipulations were performed by trained personnel and field procedures caused the minimum possible adverse effects on aquatic wildlife of the sampling sites.

Study area

The experiment was conducted in Llémena Stream (Figure 1), a tributary of the Ter River in the northeast of the Iberian Peninsula. Llémena Stream is a small calcareous stream (32 km length) with strong variation in discharge depending on rainfall. Seasonal rainstorms occur mainly during the autumn and spring (annual average 700– 900 mm year^{-1}, Catalan Water Agency, ACA), and they usually cause floods. Summer drought can substantially reduce stream flow. This catchment (drainage area 185 km^2) is characterized by a typical coastal Mediterranean climate, where streams usually maintain permanent flow. However, the presence of a weir for irrigation in the study reach caused further flow reductions downstream and intermittency in summer with the appearance of a few isolated pools, thereby increasing the duration and magnitude of drought. Similar to what has been described in other productive Mediterranean streams (e.g. Power, 1992; Power et al. 2009), algal growth is strongly influenced by the *Cladophora glomerata* cycle. Cladophora initiates growth (vegetative from basal cells) in spring, reaching maximum biomass (long attached turfs) in late spring or early summer, when it becomes thickly overgrown with epiphytes. These turfs detach to form floating mats that collapse by late summer and early autumn. Loosely attached filaments are scoured by the onset of autumn and winter rains, being replaced by diatoms that form epilithic biofilms.

Preliminary electrofishing with multiple passes (three pass depletion) and block nets indicated that the fish assemblage was dominated by Mediterranean barbel (>90% of captures) with similar size structure in both reaches (Table 1). Barbel density was relatively low, but upstream from the weir had almost threefold greater density than downstream (mean ± SE; 6447 ± 494 and 2100 ± 840 ind ha^{-1}, respectively). During the study period, both reaches contained similar habitats and were flanked by riparian vegetation, mainly alder (*Alnus glutinosa*), hazel (*Corylus avellana*), plane tree (*Platanus* × *acerifolia*), and cottonwood

(*Populus nigra*). Substrate consisted of gravel, silt and sand from Cenozoic and slates from Palaeozoic. High nutrient loads from agriculture and urban activity (Sant Gregori village, 3500 inhabitants) in the surrounding catchment of Llémena stream cause eutrophication (see Table 1).



F I G U R E 1 Location of Llémena Stream (left bottom) and map (left top) of the study area. Location of the two reaches differing in flow regime (permanent reach and regulated reach) and diagram of the experimental setup with the cages used to establish different fish densities (right)

TABLE 1 Physical and chemical features and fish size structure at the two sampling reaches from Llémena Stream

	Upstream from the weir (Permanent reach)	Downstream from the weir (Regulated reach)
Water discharge ($m^3 h^{-1}$)	62.30 ± 20.31	42.39 ± 42.45
Width (m)	8.50 ± 0.50	6.33 ± 2.02
Depth (cm)	29.86 ± 2.16	25.66 ± 0.92
Physical and chemical parameters		
Temperature (°C)	12.17 ± 0.51	12.93 ± 0.81
Oxygen (mg L^{-1})	11.14 ± 0.67	11.20 ± 0.17
Conductivity (µS cm^{-1})	463.67 ± 2.31	517.00 ± 39.85
pH	8.43 ± 0.28	8.52 ± 0.04
Light (µE $cm^{-2} s^{-1}$)	430.72 ± 199.21	718.67 ± 164.05
NO_3 (mg L^{-1})	2.05 ± 0.32	3.02 ± 1.45
PO_4 (µg L^{-1})	316.40 ± 2.50	329.6 ± 10.00
NH_4 (µg L^{-1})	19.40 ± 8.58	36.30 ± 18.60
Suspended solids (mg L^{-1})	1.42 ± 0.78	0.60 ± 0.40
Mean fork length (mm) (range)	57.76 ± 18.70	61 ± 15.86
Barbus meridionalis	(141–27)	(96–31)

Physical and chemical features from Llémena Stream correspond to the sampling period in spring (from 15th March to 15th April 2015). Values are shown as mean ± standard error

Experimental design

Surveys were performed along two reaches of Llémena stream in early spring of 2015 under high water discharge conditions (Figure 1). Reaches were separated by 2 km and the length of each sampling reach was approximately 600 m. The two reaches included: 1) a permanent least-impacted reach, located upstream from the weir (42°00′04″ N, 2°44′05″ E), and 2) a regulated intermittent reach, located downstream from the weir (41°59′19″ N, 2°44′51″ E). The downstream reach had considerably more hydrological alteration, becoming intermittent in summer. In these two reaches, we set up a mesocosm experiment that allowed us to control fish density and quantify treatment effects on resource quantity and quality, community structure, and nutrient removal following the design of Winemiller et al. (2014). Four-sided cages were constructed of 6-mm plastic mesh supported by reinforcing steel bars and enclosed an area of

1.44 m^2 (1.2 × 1.2 × 0.7 m). This mesh size excluded most benthic fish except the smallest size classes. A simple mesh was installed along the top to prevent predation of fish by aquatic birds or mammals. The bottom of cages was filled with cobbles (250–300 mm in diameter) from each sampling reach previously to the beginning of the experiment. Cobbles were carefully collected to minimize disruption to biotic compartments. The experiment followed a generalized randomized block design (Addelman, 1969), with three replicated treatments in each of the two reaches (blocks). Treatments were established by varying fish densities in the cages, as follows: (1) cages with 10 barbels simulating high fish density (>7 ind m^{-2}), (2) cage controls with open sides, thus exposed to actual fish densities of each stream reach (<1 ind m^{-2}) and used to account for potential artefacts of the cages, and (3) cages with no fish, simulating the disappearance of fish that occurs with stream drying. The distance between replicates was approximately 200 m along each reach and cages were separated a minimum of 10 m between them to avoid interference. Barbels were caught by electrofishing (LR-24 Smith-Root Ltd. 120 V DC-0.6 A) 100-m downstream from each experimental reach, and fish (mean fork length ± SE: 72.53 ± 20.39 mm and 85.87 ± 22.76 mm, permanent and regulated reaches, respectively) were added into the treatments.

The experiment ran over 14 days in April, a time period which has been shown to be enough to allow for the detection of fish effects in previous stream experiments (e.g. Flecker, 1996; Flecker and Taylor, 2004; Rodríguez-Lozano et al. 2015). Mesocosms were sampled after one week and at the end of the experiment for periphyton biomass and uptake of ammonia and phosphate. At the end of the experiment, we also sampled benthic macroinvertebrates and took more extensive periphyton measurements.

Field sampling and laboratory procedures

Nutrient uptake

Nutrient uptake was measured two times during the experiment (after 1 and 2 weeks) in both reaches and for the different fish density treatments. On each sampling date, one cobble was randomly taken from each cage, stored separately in a dark container, and transported in stream water to the laboratory within 2 h. Substrata were minimally disturbed during transport to preserve periphyton.

Nutrient uptake was measured indoors using recirculating incubation chambers as those described in Merbt et al. (2017). Briefly, chambers were made of transparent perspex (30 × 10 × 10 cm) connected to a submergible pump (24 V) and run under constant temperature. A set of LED light panels (Led's Grow 120 W V.2) illuminated the experimental setting at 120 µE m^{-2} s^{-1} constant intensity. Ten chambers filled with 8 L of stream water were run simultaneously: nine with cobbles and the last one without a cobble, thus serving as an abiotic control. Ammonia and phosphate removal were estimated by the nutrient addition technique (O'Brien and Dodds, 2008). P-PO_4 and N-NH_4 concentrations were increased 2–5 times by adding a spike of NH_4 and PO_4 from stock solutions (1000 mg L^{-1}). Water temperature was controlled with a water bath and never exceeded stream water temperature by >2 °C. Water samples (30 mL) for analysing changes in P-PO_4, N-NH_4 and N-NO_3 were taken 10 min before and 15, 30, 60, 120 and 240 min after nutrient addition. All water samples were immediately filtered through glass fiber filters (GF/F Whatman 0.45 µm-pore size) and stored at −20 °C for subsequent analysis following colorimetric methods (APHA, 1995). Dissolved nutrient samples were analysed using an Automatic Continuous Flow Futura-Alliance Analyzer at the Nutrient Analysis Service of the CEAB-CSIC.

P-PO_4 and N-NH_4 uptake rates were calculated as the slope of the regression line (obtained by means of linear regression analysis) of the mass of P-PO_4 and N-NH_4 (y axis) versus time (x axis) and given in µmol P-PO_4 min^{-1} and µmol N-NH_4 min^{-1}, respectively. At each sampling time, the mass of P-PO_4 and N-NH_4 was calculated from the multiplication of the concentration for the remaining water volume. Uptake rates (in µmol min^{-1}), were standardized for the area colonized by the biofilms (µmol m^{-2} min^{-1}) and the corresponding algal biomass (µmol mg chl^{-1} min^{-1}). Periphyton was scraped from each cobble (see next section for more details) and total algal biomass (mg chlorophyll-*a*) determined as described below.

While nutrient uptake processes are enhanced by the nutrient addition technique (O'Brien and Dodds, 2008), it is important to note that overall changes in nutrient mass will result from the balance between removal and release processes, which may be positive, negative or nil. Negative slopes are indicative of nutrient removal, resulting from a positive balance between the processes that contribute to remove the nutrient: mainly assimilatory uptake but also adsorption, precipitation (in the case of phosphate) and dissimilatory uptake (in the case of ammonia), and those contributing to its release, mainly mineralization but also re-dissolution (in the case of phosphate); des-adsorption and nitrification (in the case of nitrate). Conversely, positive slopes are indicative of nutrient release, whereas, the lack of tendency indicates that uptake and release are almost equal, thus resulting in no nutrient uptake (zero value).

The experiment was focused on measuring uptake rates of phosphate and ammonia, however nitrate changes were also assessed as complementary information to identify whether ammonia uptake was attributable to assimilatory uptake (no concomitant increase in the concentration of nitrate) or nitrification, if nitrate increased while ammonia decreased (Merbt et al. 2011). Nitrate uptake was determined following the same procedures as ammonia and phosphate.

Periphyton

On each sampling date, we collected one cobble from each cage to estimate total biomass (ash-free dry mass, ‘AFDM’, hereafter), algal bio-mass (chlorophyll-*a*), as well as the carbon, nitrogen and phosphorus content of periphyton. Cobbles were thoroughly scraped with cell scrapers, washed with water, filtered onto GF/F Whatman filters (0.45 µm-pore size) and stored frozen. Chlorophyll-*a* was measured after extracting in 10 mL of 90% acetone for 24 h in the dark at 4 °C and sonicating for 2 min at 40 kHz. Chlorophyll-*a* concentration was determined spectrophotometrically (Jeffrey and Humphrey, 1975); AFDM was determined by filtering through a pre-dried and pre-weighed filter (GF/F Whatman 0.45 µm-pore size). After filtration, filters were placed into a drying oven (100 °C) until constant dry mass was reached. Subsequently, filters were weighed on an electronic balance (±0.1 mg) and then placed into a muffle furnace for 2 h (500 °C) to burn the organic matter. Finally, filters were reweighed to quantify the difference between mass measurements (Steinman et al. 2007). Cobble dimensions were used to standardise units per surface area (mg chlorophyll-*a* m^{-2} and g AFDM m^{-2}). For periphyton elemental analyses, the scraped samples were frozen at −80 °C for 2 h, lyophilized for 24 h and homogenized using a mortar. Nitrogen and carbon content were measured using an elemental analyser (PerkinElmer 2400) and phosphorus content was determined after digestion following the protocol described by Murphy and Riley (1962). The C:N and C:P molar ratios were calculated for each replicate.

Macroinvertebrates

Benthic macroinvertebrates were collected from five randomly chosen cobbles taken from each cage using a Surber sampler (20 × 20 cm and 250 µm mesh net). Macroinvertebrates were rinsed with water to remove debris and preserved in 70% ethanol. In the laboratory, macroinvertebrates were identified and counted under a dissecting microscope (40 ×).

Individuals were identified to family following available keys (Tachet et al. 2000), and taxa were assigned to functional feeding groups (‘FFG’, hereafter): grazers, shredders, collector-gatherers, collector-filterers and predators. After identification, samples were dried (60 °C until constant mass) to obtain the dry mass (DM, ±0.1 mg) for each replicate cage. Cobble dimensions were used to standardise units per surface area (ind m^{-2} and g DM m^{-2}).

Data analyses

To compare specific treatments, we used multiple contrasts and function “adonis”. When several measurements were obtained over time (nutrient uptake: P-PO_4 and N-NH_4); we used repeated-measures analysis of variance (ANOVA), using the “aov” function in R. To test for differences in response variables of periphyton and macroinvertebrate community structure among fish treatments and the two reaches, we used permutational analysis of variance (‘PERMANOVA’, hereafter) using distance matrices (Anderson, 2001), as implemented in function “adonis” of the “vegan” package (Oksanen et al. 2017) in R (R Core Team, 2016). Compared to other techniques, PERMANOVA has the advantages of not assuming normality or a specific probability distribution and allowing several distances and designs (e.g. two-way as our study). For PERMANOVA, we used 999 permutations and Bray-Curtis distances for the composition of macroinvertebrates (FFG biomasses) and Euclidean distances for the rest (univariate descriptors).

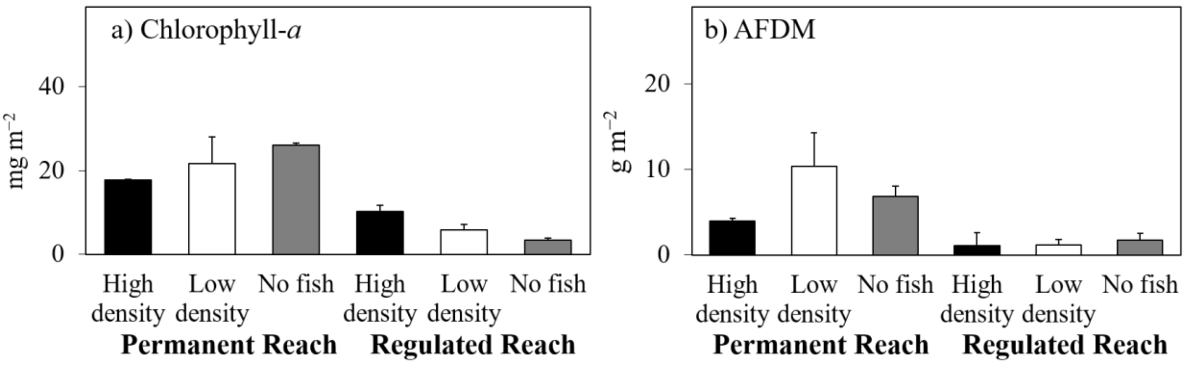
The influence of sampling reaches and fish density treatments on macroinvertebrate community structure was assessed by considering total density and biomass of the whole community; density and biomass of Ephemeroptera–Plecoptera–Trichoptera (‘EPT’, hereafter) (Barbour et al. 1998); taxa richness (S) and Simpson's diversity index (Hurlbert, 1971).

Variation partitioning (VP) was used to analyse whether macroinvertebrates composition (biomass of the five FFGs), in addition to reach and fish treatment, mediated part of the variation in response variables of periphyton (algal biomass, nutrient uptake and nitrogen and phosphorous content). VP of univariate response variables uses partial linear regression to measure variation explained uniquely and jointly by different groups of predictors (Borcard et al. 1992). We performed VP using the ‘varpart’ function of the ‘vegan’ package (Oksanen et al. 2017) in R. Significance was determined for testable fractions (variation due to unique and overall effects of each of the three predictor sets) using permutation tests (‘anova’ function with 999 permutations).

Results

Differences between reaches

Chlorophyll-*a* (Figure 2a) and AFDM (Figure 2b) were significantly greater in the permanent reach (Table 2), and the periphyton C:N ratio was lower in the regulated reach (Table 2, Figure 3a). Phosphate and ammonia uptake rates (U P-PO_4 and U N-NH_4) were not significantly different between reaches (Table 3). While U P-PO_4 values did not vary significantly with time (Figure 4a), U N-NH_4 increased (Figure 4c), particularly in the regulated reach (significant Time × Reach interaction). U P-PO_4 and U N-NH_4 per unit of chlorophyll-*a* (U P-PO_4/chl-*a* and U N-NH_4/ chl-*a*) were greater in the regulated reach (significant differences for Reach, Table 3; Figure 4b–d) and the differences increased over time (significant Time × Reach interaction, Table 3).

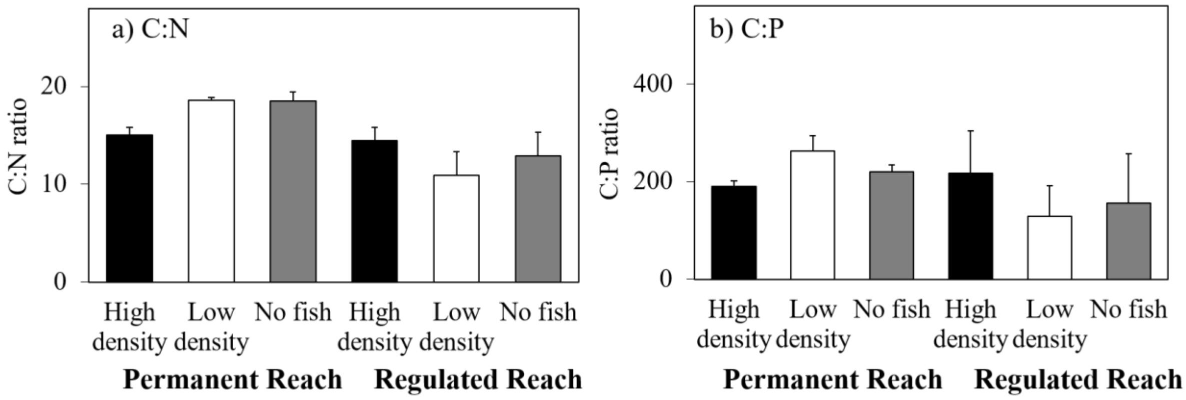


F I G U R E 2 Chlorophyll-*a* (a) and total biomass as ash-free dry mass, AFDM (b) of periphyton at the two sampling reaches. Bars represent mean ± standard error (SE). Black bars=treatments with high fish density; white bars= treatments with low fish density; grey bars = fish-less treatments

T A B L E 2 Results of two-way permutational analyses of variance: effects of reach and treatment on different response variables

Response variables	Reach			Treatment		
	R^2	SS	*P*	R^2	SS	*P*
Chlorophyll-*a* (mg m^{-2})	0.596	0.788	**0.002**	0.102	0.135	0.166
AFDM (mg m^{-2})	0.566	2.126	**0.004**	0.031	0.117	0.644
C:N ratio	0.367	50.514	**0.032**	0.039	2.719	0.679
C:P ratio	0.016	1927	0.718	0.008	971	0.942
Total biomass (mg m^{-2})	0.2	1.031	**0.001**	0.081	0.424	0.797
Total abundance (ind m^{-2})	0.198	0.521	**0.022**	0.078	0.129	0.759
EPT biomass (mg m^{-2})	0.254	0.098	**0.02**	0.072	0.028	0.552
EPT abundance (ind m^{-2})	0.324	63003	**0.018**	0.054	10635	0.636
Taxa richness (*S*)	0.016	2	0.65	0.028	1.722	0.83
Simpson diversity index	0.117	0.1	0.206	0.002	0.002	0.978

The degrees of freedom of the three sources of variation are 2 and 12 (Reach), 1 and 12, and 2 and 12 for the eight first variables and 2 and 8, 1 and 8, and 2 and 6 for the other. All Reach × Treatment interactions were not significant and are omitted from the table. Significant results ($P < 0.05$) are in boldface. FFG = functional feeding group; EPT = Ephemeroptera–Plecoptera–Trichoptera families; AFDM= ash-free dry mass



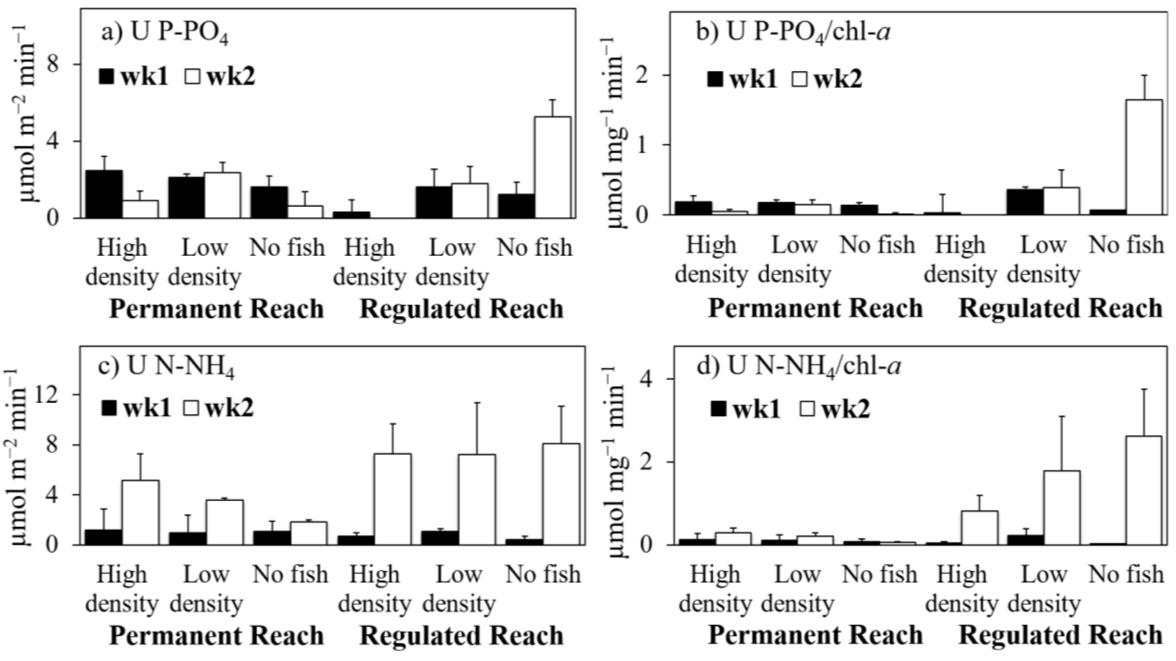
F I G U R E 3 Carbon-to-nitrogen (a) and carbon-to-phosphorus (b) ratios of periphyton at the two sampling reaches. Bars representmean±standard error. Black bars=treatments with high fish density; white bars= treatments with low fish density; grey bars= fish-less treatments

T A B L E 3 Repeated-measures ANOVAs of the effects of reach, treatment and time on uptake rate of phosphate per surface area (U P-PO_4, in µmol P-PO_4 m^{-2} min^{-1}), uptake of phosphate per chlorophyll-*a* concentration (U P-PO_4 /chl-*a*, in µmol P- PO_4 mg^{-1} chl-*a* min^{-1}), uptake of ammonia (U N-NH_4, in µmol N-NH_4 m^{-2} min^{-1}), and uptake of ammonia per chlorophyll-*a* concentration (U N-NH_4 /chl-*a*, in µmol N-NH_4 mg^{-1} chl-*a* min^{-1})

	U-P-PO_4			U-P-PO_4 /chl-*a*			U-N-NH_4			U-N-NH_4 /chl-*a*		
	Df	SS	*P*	Df	SS	*P*	Df	SS	*P*	Df	SS	*P*
Reach	1	0.06	0.407	1	0.056	**0.027**	1	0.719	0.129	1	0.229	**0.019**
Fish density	2	0.51	0.081	2	0.079	**0.033**	2	0.054	0.905	2	0.012	0.834
Reach × Fish density	2	1.004	**0.014**	2	0.102	**0.016**	2	0.112	0.815	2	0.054	0.449
Error	12	0.985		12	0.103		12	3.244		12	0.377	
Time	1	0.002	0.88	1	0.021	**0.029**	1	4.343	**0.007**	1	0.357	**0.002**
Time × Reach	1	0.42	0.073	1	0.078	**<0.01**	1	4.343	0.007	1	0.23	**0.008**
Time × Fish density	2	0.054	0.781	2	0.083	**0.001**	2	0.369	0.647	2	0.021	0.637
Time × Reach × Fish density	2	0.34	0.249	2	0.101	**0.001**	2	0.587	0.508	2	0.062	0.293
Error	12	1.309		12	0.04		12	4.9		12	0.27	

Significant results ($P < 0.05$) are in boldface

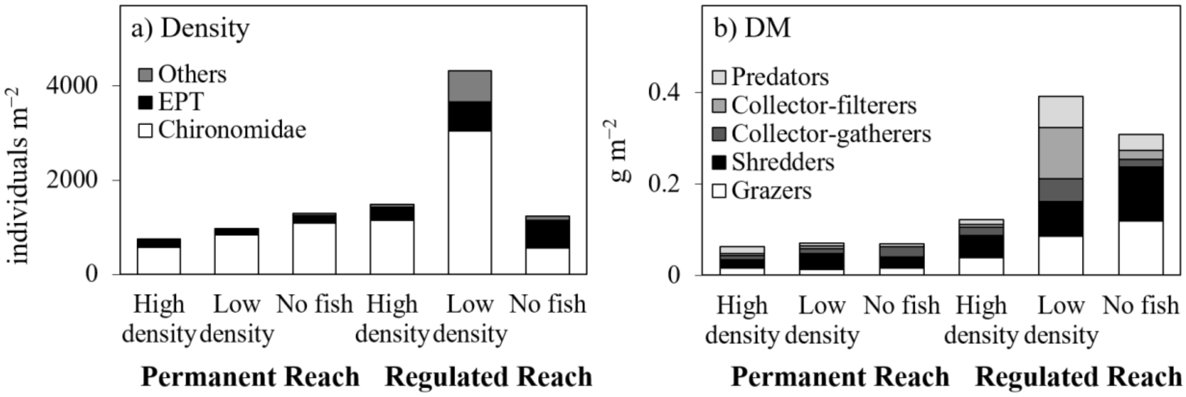
As for macroinvertebrates, thirty-two families were identified in total. The most abundant FFG was collector-gatherers, largely represented by chironomid larvae (Figure 5a), which comprised >90% of the total density in many samples (range 4.88–95.60%), followed by shredders (range 14.56–34.97%), scrapers (range 0.57–44.53%), predators (range 0–7.08%) and collector-filterers (range 0–43.84%). Taxonomic composition was dominated by Trichoptera (9 total) and Ephemeroptera (6 total) families, followed by nematoceran Diptera and Coleoptera families (4 total), Gastropoda and Odonata families (3 total) and finally, Plecoptera, Crustacea and Oligochaeta families (1 total). Four descriptors of the macroinvertebrate community (total density, total biomass, EPT density and EPT biomass) were significantly greater in the regulated reach (Table 2), where fish density was lower (Figure 5a–b).



F I G U R E 4 Phosphate uptake (a), phosphate uptake per unit of chlorophyll-a (b), ammonia uptake (c) and ammonia uptake per unit of chlorophyll-a (d) of periphyton at the two sampling reaches. Bars represent mean ± standard error. Black bars= first sampling at day 7 of the experiment (week 1); white bars= second sampling at day 14 of the experiment (week 2)

Fish density effects

Overall, there were few main effects of fish treatments for the different response variables. Treatment effects were not detected for chlorophyll-*a*, AFDM, and periphyton nutrient content (Table 2). Similarly, total macroinvertebrate density and biomass, EPT abundance and biomass, taxa richness and Simpson diversity index were not affected by the fish density treatments (Table 2).



F I G U R E 5 Density of Chironomidae, EPT taxa and other macroinvertebrate families (a) and dry mass, DM of the five functional feeding groups (b) at the two sampling reaches. EPT = Ephemeroptera–Plecoptera–Trichoptera families

Ammonia uptake was not significantly influenced by fish density. In contrast fish effects on U P-PO_4 were marginally significant ($P < 0.1$). Moreover, U P-PO_4 was statistically significant for the interaction Reach × Fish density (Table 3), since treatment effects were mainly observed at the regulated reach (Figure 4a). For its part, U P-PO_4/chl-*a* was statistically significant for Fish density showing an overall effect of this treatment. In addition, the interactions Reach × Fish density, Time × Fish density and Time × Reach × Fish density were statistically significant U P-PO_4/chl-*a* (Table 3) since fish density effects were greater in the regulated reach, increasing from week 1 to week 2 at this reach (Figure 4b).

Variation partitioning analysis

Benthic macroinvertebrates, measured as biomass of the five FFGs, explained a large part of the variation for a number of variables. FFG alone explained 30% of the variation for AFDM, 25% of the variation for U N-NH_4/chl-*a* and 44% of the variation for C:N ratio. Moreover, Reach and FFG explained 41% of the variation for AFDM, 14% of variation for U P-PO_4/chl-*a*, 26% for U N-NH_4/chl-*a* and 44% for C:N (Figure 6). Variation for FFG and the variation shared for Reach and Fish Density were significant for AFDM and C:N ($P < 0.05$), and marginally significant for U N-NH_4/chl-*a* ($P < 0.1$). Reach alone was only significant for chlorophyll-*a* and AFDM, which explained 43% and 25% of the variation, respectively (Figure 6a). Variation for Reach and the variation shared for Reach and FFG were significant for U P-PO_4/chl-*a* and C:N ($P < 0.05$ and $P < 0.01$, respectively). Fish density explained little variation in the response variables; only for U P-PO_4/chl-*a*, where the effects were marginally significant ($P < 0.1$) with 18% of the variation (Figure 6b).

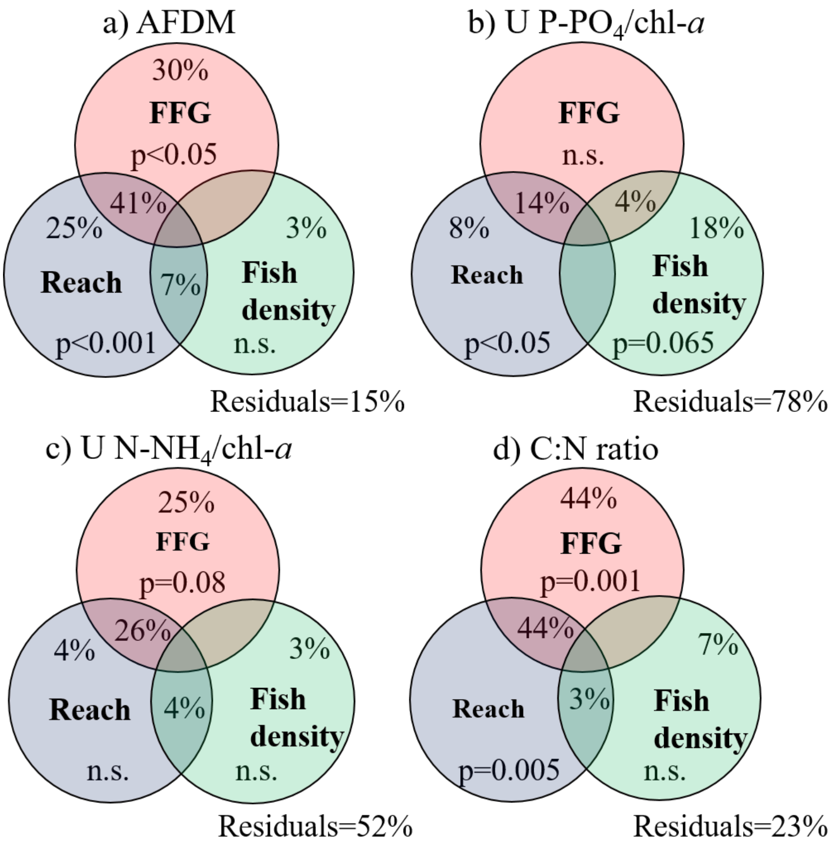


FIGURE 6 Variation partitioning of ash free dry mass, AFDM (a), phosphate uptake per unit of chlorophyll-*a*, U P-PO4/chl-*a* (b), ammonia uptake per unit of chlorophyll-*a*, U N-NH4/chl-*a* (c) and C:N ratio (d) with Reach, Fish density and biomasses of the five macroinvertebrate functional feeding groups (FFG) as predictors. Values correspond to the percentage of variation explained for each fraction (values ≤ 0 not shown). Significant results are at P < 0.05, marginally significant at $P < 0.1$

Discussion

Our results support the hypothesis that hydrologic alteration causing long periods of dryness may have strong effects in the ecosystem, influencing not only periphyton and macroinvertebrate assemblages, but also their ability to respond to another alteration, which was the absence of fish in this study. Fish density treatments did not affect macroinvertebrate community nor periphyton structure. The lack of evidence concerning the control of fish on lower trophic levels may be due to the sampling period since stronger top-down effects would be expected in summer when the regulated reach dries down to a few isolated pools (Williams et al. 2003; Wesner, 2013). Secondary to hydrology, resources subsidies (e.g. increase of nutrients) can also modify the strength of trophic interactions (Davis et al. 2010; Taylor et al. 2015), suggesting that the eutrophic environment in Llémena stream could have prevented fish control. For example, Riley et al. (2004) reported strong cascading effects of fish on invertebrates and algae in a New Zealand stream with low ambient nutrient concentrations, which disappeared under experimental conditions of higher nutrient concentrations.

Although in our experiment fish treatments displayed no measurable effects on community structure, fish influenced the capacity of periphyton to remove nutrients (i.e. uptake of phosphate), and these effects were markedly observed in the regulated reach. Thus, differences in the functional response detected could be tentatively attributed to the prevailing ecological differences (i.e. macroinvertebrate and periphyton communities) between reaches, since their biotic communities were influenced by flow regulation.

Large differences in macroinvertebrate structure were observed between reaches. The regulated reach had significantly greater macroinvertebrate abundance and biomass, differing from other studies that found greater density, biomass and production in perennial than in ephemeral and intermittent river reaches (Feminella, 1996; Smith et al. 2003; Arscott et al. 2010). Bonada et al. (2007) found similar richness between permanent and intermittent sites, but higher richness than in ephemeral sites. Moreover, chironomid larvae, more abundant in the regulated reach, were completely dominant in the macroinvertebrate community followed by EPT taxa throughout fish density treatments and stream reaches. Chironomid larvae are resilient species, highly tolerant to environmental fluctuations (Acuña et al. 2005). In our study, macroinvertebrate abundance was markedly low, compared to studies performed in similar streams (e.g. Rodríguez-Lozano et al. 2015). The low densities observed in our study could be explained by the sampling period in early spring, when both reaches were recovering from a recent flood.

Periphyton biomass showed an opposite trend to the pattern observed for macroinvertebrate biomass, indicating that herbivorous consumers were able to control algal biomass by intense grazing pressure (Feminella and Hawkins, 1995; Hillebrand et al. 2002; Barbee, 2005; Guasch et al. 2016). As a consequence, the remaining epilithic biomass was more nutrient-rich (lower C:N ratio) in the regulated reach. These results are consistent with the notion that macroinvertebrate assemblages (e.g. scrapers) may enhance biomass-specific productivity by removing dead or senescent algal cells (rejuvenating effect); allowing light and nutrient penetration and remobilizing nutrients, as reported by other authors (Bothwell, 1988; Mulholland et al. 1991; Wallace and Webster, 1996; Evans-White and Lamberti, 2005; Guasch et al. 2016). Moreover, the variation partitioning results support this hypothesis since macroinvertebrate assemblages as functional feeding groups largely explained the variance of periphytic AFDM and C:N ratio.

For its part, nutrient removal by periphyton was similar in both reaches. However, it applies only to phosphate. In addition to ammonia uptake, nitrate uptake was also detected in the permanent reach. It was on average 36 ± 16 µmol N NO_3 m^{-2} min^{-1} (mean ± SE) indicating an important contribution of assimilatory uptake to ammonia removal. Nitrate assimilation was not common within the regulated reach. On the contrary, ammonia uptake was associated in many cases with the release of nitrate, leading to average negative nitrate uptake values (−30 ± 28 µmol N-NO_3 m^{-2} min^{-1}, mean ± SE). This suggests an important contribution of nitrification to ammonia removal in this reach. These differences may be attributed to episodes of high ammonia concentration (Merbt et al. 2011), which are more likely to occur under low flow conditions (Martí et al. 2001; Merseburger et al. 2005).

As mentioned above, the exclusion of barbels (i.e. no fish treatment) in the regulated reach resulted in higher phosphate uptake by periphyton at the end of the experiment: 30 times higher than in the fish density treatment and two times higher than in the low density treatment (Fig. 4a). This increase was not due to higher biomass but the contrary, algal biomass was slightly lower leading to an even much higher increase in phosphate uptake per chlorophyll unit (Fig. 4b). Fish exclusion may have had an indirect effect on phosphate uptake by increasing grazing pressure (Haglund and Hillebrand, 2005). Similarly to the differences observed between reaches, higher grazing pressure may result in higher phosphate uptake of a rejuvenated and more active periphyton community. However, this effect was not experimentally demonstrated since macroinvertebrate densities were not released in treatments lacking fish. This apparent contradiction may be related to the duration of the experiment, which may have been long enough to see effects on functional metrics (i.e. nutrient uptake), but not to cause detectable structural changes (Corcoll et al. 2012).

In contrast to the results obtained in the regulated reach, fish density did not affect nutrient uptake in the permanent reach. It may tentatively be concluded that the effects of our treatment were compensated by trophic interactions in the permanent reach which was likely to have more stable communities (Ghedini et al. 2015). However, when more extreme events such as extended water intermittence episodes occur, the effects of multiple disturbances may be larger than the capacity of compensatory mechanisms.

In summary, this study provides insights into effects of flow alteration on the structure and function of fluvial ecosystems that persist after channel rewetting. Hydrologic alteration effects were clearly manifested in the regulated reach when we removed fish in our treatments. While fish density treatments did not lead to changes in the structure of biotic communities (macroinvertebrates and algal biomass), phosphate uptake was enhanced in treatments lacking fish. This result suggests that less stable communities from the regulated reach, which are more exposed to hydrologic alteration over the year, could not compensate for the loss of fish. It seems that fish density can mediate the effects of anthropogenic alterations such as flow intermittence on stream benthic communities and associated biogeochemical processes, at least in eutrophic streams. It is therefore expected that the effects of hydrologic alteration on fluvial systems may be heightened if this alteration leads also to the disappearance of fish. Nevertheless, research at larger spatial and temporal scales is needed to better understand the interplay between communities and biogeochemical responses.

CHAPTER IV

Indirect food-web interactions are mediated by metal pollution and an ecosystem engineer, *Barbus meridionalis* (Cyprinidae), in a Mediterranean stream

Rubio-Gracia F., Argudo A., Zamora L., Clements W. H., Vila-Gispert A., Frederic C. and Guasch H. Indirect food-web interactions are mediated by metal pollution and an ecosystem engineer, *Barbus meridionalis* (Cyprinidae), in a Mediterranean stream. *Aquatic Sciences* (submitted)

Introduction

Chemical pollution represents one of the major threats to fluvial ecosystem integrity and biodiversity (Allan and Flecker, 1993). The occurrence of metals in fluvial ecosystems is commonly due to urban and mining activities (Geist, 2011). Metal pollution associated with mining activity can produce adverse effects on algal periphyton biomass, taxa richness and photosynthetic efficiency (Hill et al. 2000; Morin et al. 2008; Corcoll et al. 2012; Bonet et al. 2013); and on the diversity, taxa richness and abundance of benthic invertebrates (Clements et al. 2000; Maret et al. 2003; Smolders et al. 2003; Iwasaki et al. 2009). Depending on metal species and their concentrations in the environment, aquatic organisms can also accumulate metals through two basic routes: by direct absorption through water and/or by feeding (Clements and Rees, 1997; Farag et al. 1998). Because periphyton readily bioaccumulates metals and is the primary food resource for invertebrates that scrape mineral and organic surfaces (Farag et al. 1998; Goodyear and McNeill 1999), it represents a potential link in the transfer of metals to higher trophic levels (Guasch et al. 2016; Bonnineau et al. 2020). Bioaccumulation data can be then used to characterize the partitioning of water contamination and the transport of metals into the biotic community.

The co-occurrence of contaminants with other anthropogenic stressors, such as hydrological alterations that cause water stress, is common in freshwater ecosystems (Ormerod et al. 2010). It has been shown that water diversion exacerbates the ecological impacts of metal pollution (Guasch et al. 2009, 2010; Arenas-Sánchez et al. 2016), as the capacity to dilute pollutants is compromised (Petrovic et al. 2011). Recent studies have yielded invaluable information on the direct and combined effects of chemical pollution and water stress on target organisms, such as periphyton and invertebrates (Ponsatí et al. 2016; Sabater et al. 2016; Kalogianni et al. 2017; Karaouzas et al. 2018). However, biotic interactions within food webs, such as trophic interactions (predation and grazing) and non-trophic interactions (competition for space and recruitment facilitation) can modify stressor effects, transfer stressor effects to distant groups of organisms, and create new stressor interactions (Bruder et al. 2019). For example, several field and theoretical studies have demonstrated contaminant-induced changes in either behaviour (Schmitz et al. 2004; Saaristo et al. 2018), competition or predation/grazing rate (Fleeger et al. 2003; Clements and Rohr, 2009; Alexander et al. 2013; Rodrigues et al. 2018) that altered species abundances or community composition. As illustrated in Figure 1, the effects of contaminants and other stressors can alter ecological outcomes by affecting a specific trophic level, and/or by modifying the strength of biotic interactions. Therefore, accounting for biotic interactions within food webs can increase our mechanistic understanding of the direct and indirect effects of environmental stressors (Clements and Rohr, 2009; Segner et al. 2014; Brooks and Crowe, 2018; Bruder et al. 2019).

Community ecologists recognize trophic cascades as the effects of predators that propagate downward across trophic levels through indirect interactions (Wootton, 1994; Polis et al. 2000; Ripple et al. 2016). Typically, the manipulation of the top trophic level (often exclusion) results in cascade responses, such as a decrease in periphyton biomass due to increased grazing pressure in the absence of insectivore fish (Flecker and Townsend, 1994; Biggs et al. 2000; Rosenfeld, 2000). In systems with invertebrate predators (Figure 1), the absence of fish may lead to a decrease in grazer abundance with a subsequent increase in periphyton biomass (Brashares et al. 2010). However, not all food webs have a linear shape (Rodríguez-Lozano et al. 2015) and consumer effects are often context dependent (Holomuzki et al. 2010). One driver that may regulate trophic cascade strength within systems is environmental stress, which is well known to mediate biotic interactions (Menge and Sutherland, 1987). In 1987, Menge and Sutherland developed the environmental stress model, in which top-down community regulation is predicted to dominate in benign habitats, whereas stressful environmental conditions can attenuate consumer-prey interactions (e.g. due to predator emigration) and thereby alter trophic cascades. Since Menge and Sutherland's work, a growing number of studies has been focused on quantifying the strength of trophic cascades across environmental stress gradients, especially in salt marshes (e.g. Korpinen et al. 2007; Cheng and Grosholz, 2016; McAfee and Bishop, 2019), but with a limited number of studies performed in streams (e.g. Layer et al. 2010). Habitat complexity, resource subsidies, the type and intensity of environmental stress, predator behaviour and efficiency, and system productivity are known to influence interactions within food webs (Brönmark et al. 1997; Borer et al. 2005; Leroux and Loreau, 2008; Klemmer and Richardson, 2013).

Regarding the effects of metal pollution, previous studies found that the relative importance of predation can be altered due to changes in prey immigration (Clements, 1999) or in background prey density (Kiffney, 1996). However, these studies were focused on the impacts of predatory stoneflies (Plecoptera: Perlidae) on invertebrate community structure using laboratory and stream microcosms. Yet, how metal pollution and predatory fish influence top-down control of primary producers still remains largely unknown.

This study was conducted in the Osor stream (Catalonia, Spain). This stream features a metal pollution gradient as a result of metal inputs from mine drainage and runoffs, and water diversion (Tlili et al. 2011; Corcoll et al. 2012; Bonet et al. 2013, 2014). This pollution gradient provides a unique opportunity for understanding how trophic relationships interact with environmental stress, and to test whether the ecological role of top predators in streams is context dependent. Therefore, we aimed to evaluate the responses of periphyton biomass and abundance of benthic macroinvertebrates to the presence and absence of a predatory fish along the metal pollution gradient. To address this, we used an insectivore benthic species (Mas-Martí et al. 2010; Rodríguez-Lozano et al. 2016b), the Mediterranean barbel (*Barbus meridionalis*), as a study case in a field exclosure/enclosure mesocosm experiment. We hypothesised that (i) effects of metal pollution would negatively impact stream benthic communities (Bonet et al. 2013); (ii) the absence of the top predator (*B. meridionalis*) would promote community-level cascades in benign or low altered environments (Rodríguez-Lozano et al. 2016a), whereas trophic cascade strength would vary predictably as a function of environmental stress (Cheng and Grosholz, 2016); and (iii) the direct negative effects of metal exposure on a specific trophic level may trigger indirect positive effects on other trophic levels (Fleeger et al. 2003; Clements and Rohr, 2009).

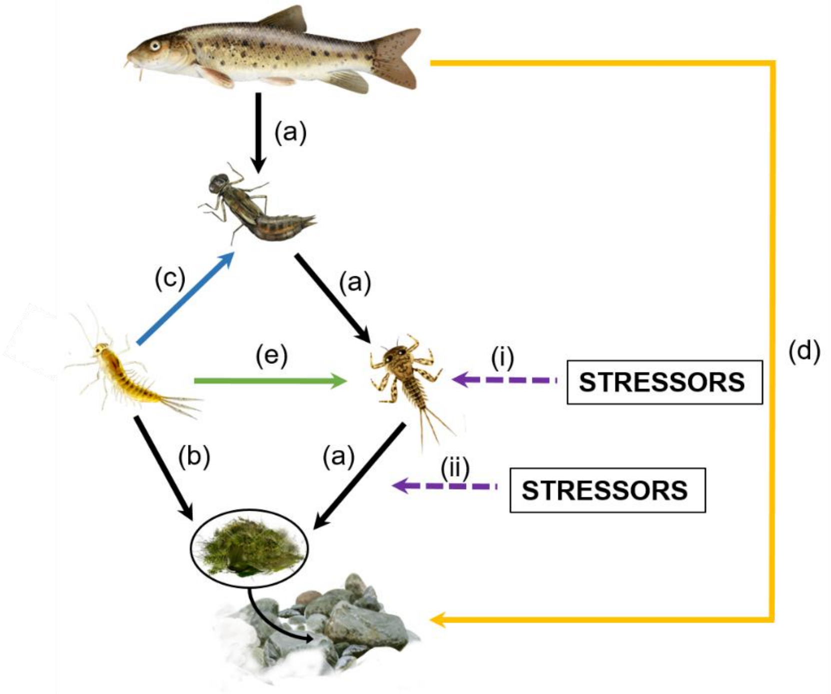


FIGURE 1 A simplified representation of biotic interactions in a stream food web (with four trophic levels) based on periphyton with the presence of stressors (e.g. contaminants). Black arrows (a,b) represent trophic interactions with top-down effects on resources, with (b) representing an organism with certain degree of omnivory. The blue arrow (c) represents a trophic interaction with bottom-up effects on the apex consumer. The orange arrow (d) represents an indirect consequence of trophic interactions on periphyton biomass, such as cascading effects (consumptive and nonconsumptive effects), whereas the green arrow (e) represents non-trophic interactions (competition or facilitation) among species in the same trophic level. Purple dashed arrows represent effects of contaminants on organism groups (i) and on interactions (ii), e.g., due to changes in foraging behavior. Modified from: Bruder et al. (2019)

Methods

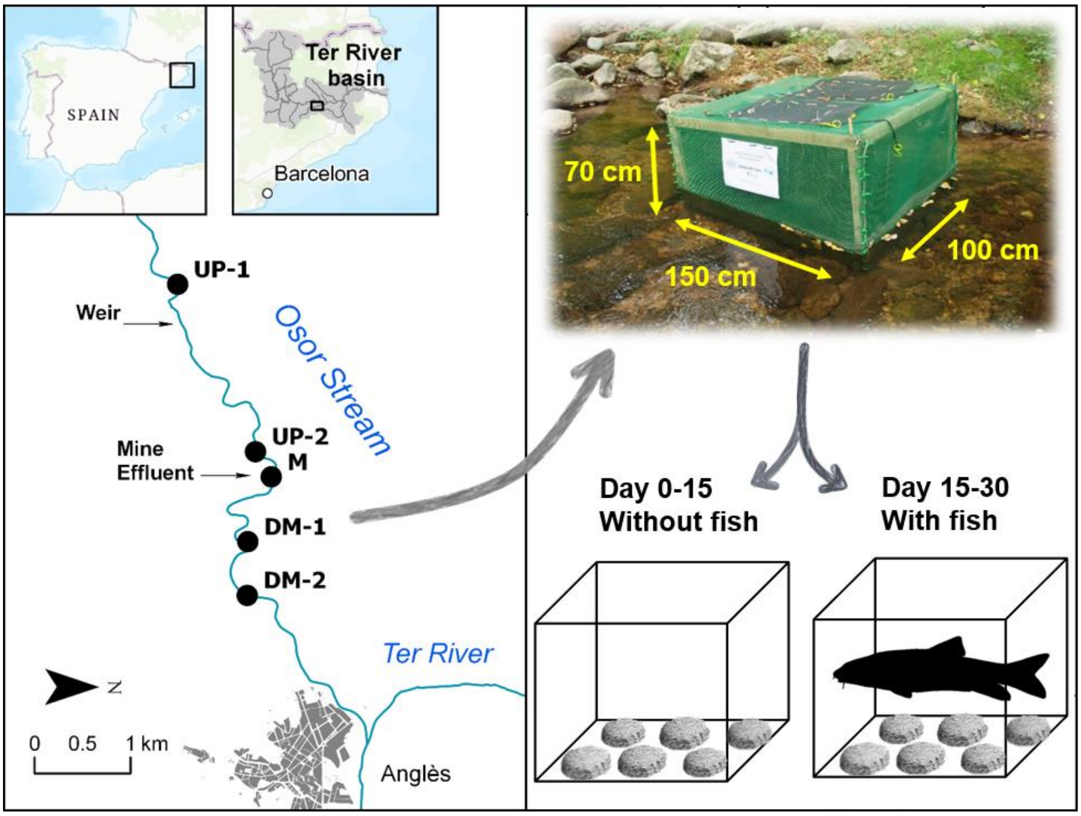
Study area

This study was carried out in Osor stream, a second order stream in the northeast of the Iberian Peninsula, Spain (Figure 2). This stream is 23.5 km long, with a drainage area of 88 km^2, and runs primarily through Guilleries Mountains until drains into the Ter River. It features moderately hard waters (1 mM Ca^{2+}) and well-preserved riparian vegetation. However, several human-driven stressors such as hydrological alteration and metal pollution threaten the ecological integrity of the stream. This catchment is subjected to seasonal rainstorms during the autumn and spring, causing spates that increase stream base-flow; however, during the summer drought, the stream discharge is substantially reduced. The presence of a diversion channel causes further flow reductions downstream (from 0.38 ± 0.28 to 0.14 ± 0.18 m^3 s^{-1}, average values obtained from Catalan Water Agency), and eventually causes streambed drying (personal observation). This stream also receives effluents and runoff from an inactive former mine that extracted spharelite ((Zn, Fe) S) and galena (PbS). Dissolved concentrations of metals in water depend on season and stream discharge, with the highest concentrations coinciding with periods of summer drought (Bonet et al. 2013). Zinc is one of the most abundant elements in the surroundings of the mine discharge (nearly 450 µg Zn L^{-1}), and concentrations often exceed the water quality standards stipulated by European and American legislation (Bonet et al. 2014).

Five sampling reaches were selected along 5-km segment of the stream with the purpose of obtaining a metal pollution gradient (Figure 2). These reaches were: Upstream 1 (UP-1; the reference site), located at about 3.7 km distance away from the mine effluent and unaffected by water abstraction; Upstream 2 (UP-2), located 100 m upstream of the most polluted reach and altered by the presence of a diversion channel for hydropower production; Mine (M), located just down the mine discharge and with the highest metal concentrations in water;

Downstream 1 (DM-1), located at about 0.6 km downstream from the mine discharge and altered by water abstraction and diversion; and finally Downstream 2 (DM-2), located at 1.4 km downstream from the mine and considered partially recovered from the diverted flow.

Preliminary electrofishing with multiple passes (three pass depletion) and block nets indicated that the fish assemblage was largely dominated by the native Mediterranean barbel (*Barbus meridionalis*) and the invasive minnow (*Phoxinus sp.*). The mean densities (individuals ha^{-1}) of *Barbus meridionalis* and *Phoxinus sp.* along the selected reaches were, respectively: 2164 and 2305 in UP-1; 1986 and 2416 in UP-2; 1362 and 3715 in M; 2419 and 3523 in DM-1; and 4115 and 1652 in DM-2.



F I G U R E 2 Localization of the five selected reaches along the Osor stream (NE Iberian Peninsula, Ter river basin), and diagram of the experimental setup showing the size of the mesocosms (cages) and the two treatments established

Description of water chemistry, periphyton and macroinvertebrates

In summer 2017, several samplings were conducted within each study reach to describe the direct effects of environmental stress on biological communities (periphyton and benthic macroinvertebrates). A field mesocosm experiment was performed in parallel to test the influence of fish on the reported responses (described in section 2.3).

Water chemistry

Physicochemical parameters were measured in-situ using a multi-parametric probe (WTW Meters, Weilheim, Germany). Water samples were taken for nutrient and dissolved metal analysis. Phosphate and ammonium concentrations were measured colorimetrically (Murphy and Riley 1962; Reardon et al. 1966) using a spectrophotometer (Shimadzu UV-1800). Analytical methods for trace metal concentrations are described in section 2.4.

Periphyton sampling

Cobbles were randomly collected in the field and were thoroughly scraped with cell scrapers, rinsed with water and filtered onto GF/F Whatman filters (0.7 µm-pore size). In the laboratory, samples were stored frozen at –20 °C and aliquots of these samples were used to estimate chlorophyll-*a* concentration and total organic biomass or ash-free dry weight (AFDW). Chlorophyll-*a* was measured after extracting in 10 mL of 90% acetone for 24 h in the dark at 4 °C and sonicating for 2 min at 40 kHz. Chlorophyll-*a* concentration was determined spectrophotometrically (Jeffrey and Humphrey, 1975). Ash-free dry weight (AFDW) biomass was determined following standard methods (Steinman et al. 2007): GF/F Whatman filters (0.7 µm-pore size) were dried at 50 °C for 48 h, weighed to determine dry weigh, combusted at 450 °C for 4h to determine mineral content, and reweighed to determine the ash-free dry weight (AFDW); estimated as the difference between weight measurements. Cobble dimensions were used to standardise periphyton biomass per surface area (µg chlorophyll-*a* cm^{-2} and mg AFDW cm^{-2}). Samples of periphyton were also taken (one sample per reach) for analysis of metal bioaccumulation (see metal analysis section) and were stored frozen at –20 °C. We calculated the bioconcentration factor (BCF) as the ratio of metal concentration in periphyton to that in water, considering that metal toxicity for organisms depend on bioavailability in the abiotic medium and the capacity of organisms to accumulate metals.

Macroinvertebrates sampling

Benthic macroinvertebrates were collected from undisturbed shallow runs and riffles using a Surber sampler (20 × 20 cm and 250 µm mesh net) and were stored in plastic bottles containing 70% ethanol. We used eight replicates for each sampling site because this amount of sampling effort is considered sufficient to characterise macroinvertebrate communities (Gartzia De Bikuña et al. 2015). In the laboratory, benthic macroinvertebrates were counted and identified to family under a dissecting microscope. Families of mayflies (Ephemeroptera), stoneflies (Plecoptera) and caddisflies (Trichoptera) were grouped as EPT taxa, whereas families of dragonflies (Odonata), beetles (Coleoptera) and true bugs (Hemiptera) were grouped as OCH taxa. Following Tachet et al. (2000), macroinvertebrate families were assigned to functional feeding groups (FFG): grazers, shredders, collector-gatherers (also termed deposit-feeders), collector-filterers and predators. For taxa assigned to multiple FFGs, densities were divided evenly into each applicable FFG (estimated as a percentage). Parasites were omitted from the analysis. After identification, samples were dried at 60 °C until constant mass and weighed (± 0.1 mg) to obtain the total dry mass (DM). For snails, shells were removed and the remaining body parts were dried and weighed. Density of macroinvertebrates and total dry mass (all individuals pooled) were standardised by surface area sampled (individuals m^{-2} and g DM m^{-2}). A sample of four representative macroinvertebrate families (Leuctridae, Hydropsychidae, Gomphidae and Lymnaidae) was also used for analysis of metal bioaccumulation. In total, we collected a minimum of 50 individuals per family from each reach. Samples included a mixture of taxa within each family to avoid interspecific variability in metal accumulation and to obtain sufficient biomass for metal analysis (Kotalik and Clements, 2019). Macroinvertebrates were stored in plastic vials and frozen at –80 °C until metal analysis (see metal analysis section). The bioaccumulation factor (BAF) was calculated as the ratio of metal concentration in macroinvertebrates to that in periphyton.

Exclosure/enclosure mesocosm experiment

Following previous work (Rubio-Gracia et al. 2017), we set up a mesocosm experiment that allowed us to control fish density and quantify treatment effects on trophic levels. The experiment was conducted for 4 weeks between June and July 2017. We established two treatments (exclosure and enclosure) replicated three times in each study reach with a duration of 2 weeks each one (Figure 2). Treatment effects were evaluated following a before-and-after design: (1) firstly, cages without fish were used to simulate the lack of fish predation (exclosure experiment), and (2) afterwards, 6 *Barbus meridionalis* were placed into the same cages (enclosure experiment), simulating the fish density (around 6 individuals per m^2) that can be found in well-preserved Mediterranean rivers. The 90 barbels used in this experiment (range fork length, FL = 84–115 mm) were previously caught by electrofishing (LR-24 Smith-Root Ltd. 120 V DC-0.6 A) from the reference site (Upstream 1) and transferred to downstream reaches. Thus, we avoided the potential harmful effects of long-term metal bioaccumulation on fish. Mesocosms (150 × 100 × 70 cm) consisted of four-sided cages made of high-density polyethylene (HDPE), which were held up by a wooden square structure. Cages were separated a minimum of 10 m to avoid interference. The mesh size of cages (4.5 mm-pore size) retained or excluded fish but allowed macroinvertebrate movement and algal colonization, thereby minimising the impact of our experimental design on trophic interactions. To simulate natural conditions and promote rapid colonization by stream communities, cage bottoms were filled with cobbles taken from the same sampling point. The substrate used during the experiment was returned to the stream channel to avoid repetition of sample units.

Surveys were conducted after 1 and 2 weeks over the course of each treatment period. On each sampling date, three randomly selected cobbles were hand-lifted, and their surfaces carefully rubbed to dislodge any aquatic macroinvertebrates, while one random boulder was used to sample the periphytic algae. We essentially followed the same methodology described above for the determination of macroinvertebrate densities, chlorophyll-*a* concentration and AFDW biomass.

Metal analysis

Metal analyses included water, periphyton and macroinvertebrate samples. Water samples were filtered onto Whatman nylon filters (0.2 µm-pore size) and acidified with 1% HNO_3 (65% suprapure, Merck). Dissolved metals were determined by inductively coupled plasma mass spectroscopy (ICP-MS 7500c Agilent Technologies). Periphyton and macroinvertebrate samples were processed and analysed for Zn, Mn, Fe, Cr, Cd, Cu and Pb. All samples were lyophilized, weighted with an analytical balance scale (Menttler-Toledo AX205), and digested in nitric acid (4 mL HNO_3, 65% suprapure, Merck) and hydrogen peroxide (1 mL H_2O_2, 30% suprapure, Merck) in a high performance microwave digestion unit (Milestone, Ethos Sel). Digested samples were analysed by inductively coupled plasma mass spectroscopy (ICP-MS 7500c Agilent Technologies), and digestates for Zn from macroinvertebrates were analysed by inductively coupled plasma mass (ICP-OES 5100 Agilent Technologies). Analytical accuracy was determined using certified reference material of the Joint Research Centre (European Commission), i.e., standard trace elements in fish muscle tissue (ERM-BB422).

Data analysis

Statistical analyses were performed in the R software environment (R Development Core Team, 2018). Residual plots of response variables were used to test for the homoscedasticity and normality of residuals. Generally, response variables of the descriptive study followed a normal distribution after applying the square root transformation. If parametric assumptions were met, analysis of variance (ANOVA) was used to test for differences in response variables among stream reaches. If not, non-parametric test (Kruskal-Wallis test) was then used as an alternative to the one-way ANOVA. Tukey and Dunn's tests of multiple comparisons were subsequently used to elucidate significant differences among individual reaches. Linear regressions were applied to predict the relationship between metal concentrations in periphyton and macroinvertebrate families, while intercepts of parallel regression lines were compared using analysis of covariance (ANCOVA). Further, we performed pairwise comparisons of adjusted means (Bonferroni method) using the 'emmeans' package in R to identify which macroinvertebrate families were different. Regarding the experimental study, macroinvertebrate and periphyton data was better transformed into normality and homogeneity using the Box-Cox power transformation (Box and Cox, 1964). The optimal exponent (lambda) for each dependent variable was estimated by using maximum likelihood estimation through regression models with 'Reach' and 'Treatment' as predictors. Since the Box-Cox Power transformation only works if all the data is greater than zero, a fixed value ($\alpha = 0.5$) was added to dependent variables before applying the transformation. Due to the low densities of some macroinvertebrate orders in cages, we used the density of EPT and OCH metrics in order to increase the statistical power of subsequent linear models. The transformed responses were then fit using linear mixed models (the 'lmer' function of the 'lme4' package in R; Bates et al. 2015) to investigate the interaction effects between 'Reach' and 'Treatment' as fixed factors, while 'Time' and 'Replicate' were treated as random factors. We tested the random-effect terms in the model using the 'ranova' function of the 'lmerTest' package in R (Kuznetsova et al. 2017). Finally, we calculated *P* values and the marginal and conditional R^2 with the 'lmerTest' and 'MuMIn' R-packages (Nakagawa and Schielzeth, 2013), respectively. The marginal R^2 describes the variance explained by the fixed effects, while the conditional R^2 describes the variance jointly explained by the fixed and the random effects.

Results

Characterization of water chemistry and stream communities

Physicochemical parameters of the sampling reaches are presented in Table 1. Water discharge was markedly lower in the reach located just downstream the diversion channel, i.e., Upstream 2, and there was a progressive recovery of stream discharge in downstream reaches. We also found that nutrient concentrations (phosphate and nitrate) were slightly higher in upstream reaches than in the Mine and downstream reaches. Metals such as Zn, Mn and Fe largely showed the highest concentrations in water (Table 1) and in periphyton (Table 2). There were no significant differences in total biomass (AFDW) and chlorophyll-*a* concentration among reaches (Kruskal-Wallis test, $P > 0.05$). Periphyton showed the highest concentrations of mining metals in Mine reach, followed by Downstream 1 and 2, Upstream 2, and finally Upstream 1 (Table 2).

In total, forty-nine families of macroinvertebrates were identified in Osor stream. Family richness was similar among reaches (Table 1); however, we found significant differences in total macroinvertebrate density (ANOVA; $F_{4, 10} = 5.43$, $P = 0.014$) and total dry mass ($F_{4, 10} = 8.50$, $P = 0.003$) between Upstream 2 and both Mine and Downstream 1 (Table 1; Figure 3a). In the least polluted reaches, the macroinvertebrate community was dominated by Plecoptera (mostly Leuctridae), followed by Coleoptera (mostly Elmidae), Trichoptera (mostly Hydropsychidae, Polycentropodidae and Philopotamidae), and nematoceran Diptera (mostly

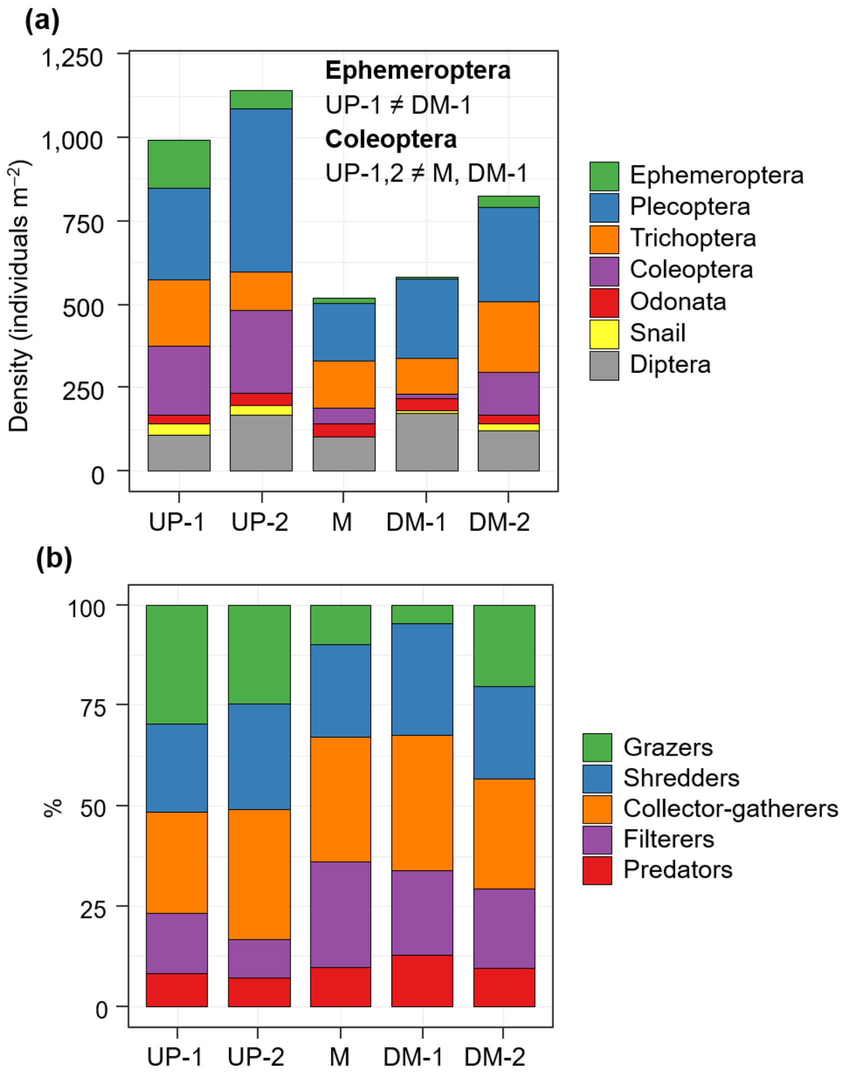
Chironomidae). In contrast, Ephemeroptera (mostly Caenidae, Ephemerillidae and Baetidae), Odonata (mostly Gomphidae) and snails (mostly Ancylidae and Lymnaeidae) were generally less abundant along the stream, especially in the most polluted reaches (Figure 3a). There were also very few annelids (Oligochaeta) in the stream (<15 individuals m^{-2}). We found that densities of Coleoptera were significantly lower in Mine and Downstream 1 (ANOVA; $F_{4, 10} = 16.43$, $P < 0.001$), and densities of Ephemeroptera were significantly lower in Downstream 1 (Kruskal-Wallis test; chi-squared = 11.31, df = 4, $P = 0.02$). Overall density of grazers was significantly lower in Mine and Downstream 1 (ANOVA; $F_{4, 10} = 19.56$, $P < 0.001$), so that the density of this functional feeding group accounted for a small proportion of the total macroinvertebrate density in those reaches (Figure 3b).

As with periphyton, macroinvertebrates mostly bioaccumulated Zn, Mn and Fe, and it was also found that periphyton showed much greater metal concentrations compared to macroinvertebrates (Table 2). An increase in concentration from periphyton to grazers of the family Lymnaeidae (BAF >1) was only observed for Cu in all reaches where these snails were found (Table 2). The relationship of metal concentrations between periphyton and macroinvertebrates was significant for several metals (Zn, Pb, Cr, and Cd) in two or more macroinvertebrate families (Table S1). Therefore, this shows that periphyton and macroinvertebrates, although having different metal loads, followed similar patterns of metal bioconcentration and bioaccumulation across the pollution gradient. Moreover, we found differences in bioaccumulation of Zn (ANCOVA, $F_{3, 13} = 6.15$, $P = 0.008$), Pb ($F_{3, 13} = 18.64$, $P < 0.001$) and Cd ($F_{2, 11} = 4.88$, $P = 0.03$) among the macroinvertebrate families studied. Concentrations of Zn and Pb in invertebrates increased along the gradient Leuctridae = Hydropsychidae > Gomphidae (Figure 4a-b), at similar periphyton metal concentrations; however, concentrations of Cd in Leuctridae were higher than in Gomphidae, at similar periphyton Cd concentrations (Figure 4c).

TABLE 1 Physical and chemical features of water, and community structure of periphyton and macroinvertebrates along the Osor stream

	UP-1	UP-2	M	DM-1	DM-2
Physicochemical characteristics					
Water discharge (m^3 s^{-1})	0.12 ± 0.02	0.05 ± 0.01	0.08 ± 0.03	0.06 ± 0.01	0.09 ± 0.01
Temperature (°C)	18.9 ± 1.5	20.14 ± 1.6	20.96 ± 1.7	20 ± 1.2	20.76 ± 1.0
Oxygen (mg L^{-1})	8.37 ± 0.46	7.75 ± 0.29	8.09 ± 0.24	8.18 ± 0.1	8.18 ± 0.28
pH	8.34 ± 0.08	8.14 ± 0.06	8.19 ± 0.07	8.24 ± 0.16	7.86 ± 0.13
Cond (µS cm^{-1})	373 ± 41	416 ± 82	517 ± 25	484 ± 18	463 ± 30
$P - PO_4^{3-}$ (mg L^{-1})	0.53 ± 0.11	0.42 ± 0.04	0.30 ± 0.03	0.36 ± 0.11	0.42 ± 0.09
NH_4^+ (µg L^{-1})	27.42 ± 17.01	33.54 ± 14.21	48.56 ± 44.15	25.52 ± 6.88	28.17 ± 3.91
NO_3^- (mg L^{-1})	2.59 ± 0.74	1.22 ± 0.29	1.04 ± 0.36	1.44 ± 0.58	1.52 ± 0.46
Zn (µg L^{-1})	21.02 ± 20.05	25.41 ± 10.92	349.0 ± 121.69	224.98 ± 93.48	84.61 ± 36.78
Mn (µg L^{-1})	20.82 ± 7.79	25.25 ± 8.06	143.38 ± 34.78	48.36 ± 14.18	24.68 ± 10.80
Fe (µg L^{-1})	132.94 ± 60.89	128.20 ± 152.15	96.39 ± 23.45	91.72 ± 39.96	47.16 ± 36.07
Ni (µg L^{-1})	0.83 ± 0.70	0.43 ± 0.08	3.34 ± 1.09	2.22 ± 1.26	1.55 ± 0.99
Periphyton					
Chlorophyll-*a* (µg cm^{-2})	1.08 ± 0.81	3.36 ± 2.73	2.70 ± 2.26	7.21 ± 7.06	7.02 ± 8
AFDW (mg cm^{-2})	0.84 ± 0.62	0.98 ± 0.26	0.58 ± 0.12	1.04 ± 0.58	0.97 ± 0.53
Macroinvertebrates					
Family richness	23.33 ± 3.09	21.00 ± 1.63	16.67 ± 1.70	20.00 ± 1.41	21.33 ± 3.09
Total density (individuals m^{-2})	1000.08 ± 191.57^{a,b}	1147 ± 267.22^{b}	520.06 ± 63.05^{a}	580.70 ± 99.15^{a}	835.14 ± 188.34^{a,b}
Total dry mass (g DM m^{-2})	0.41 ± 0.08^{a,b}	0.73 ± 0.10^{b}	0.21 ± 0.08^{a}	0.30 ± 0.10^{a}	0.44 ± 0.09^{a,b}

Stream reaches are: Upstream 1 (UP-1; the reference site), Upstream 2 (UP-2), Mine (M), Downstream 1 (DM-1) and Downstream 2 (DM-2). Nutrient concentrations were obtained from Argudo et al. (2020 in prep). Data are shown as average ± standard deviation ($N = 3$). Significant differences in variables of periphyton and macroinvertebrate among the stream reaches are represented by different lowercase letters (Tukey's test, $P < 0.05$)



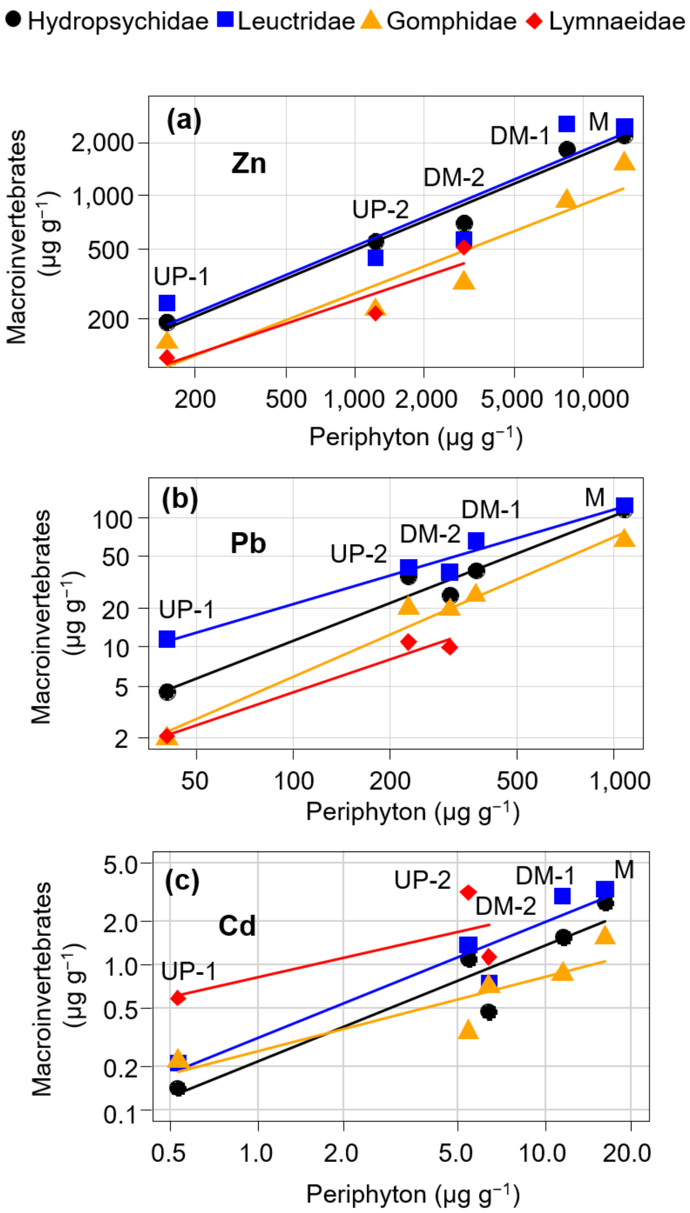
F I G U R E 3 Density of benthic macroinvertebrates (a) and proportion of the different feeding groups (b) along the Osor stream. Stream reaches are: Upstream 1 (UP-1), Upstream 2 (UP-2), Mine (M), Downstream 1 (DM-1) and Downstream 2 (DM-2). Dunn and Tukey's tests showed significant differences in the density of Ephemeroptera and Coleoptera orders among the reaches, respectively ($P < 0.05$)

T A B L E 2 Trace metal concentration (μg g^{-1}), bioconcentration factor (BCF) in periphyton, and bioaccumulation factor (BAF) in four macroinvertebrate families (stonefly, family Leuctridae; caddisfly, family Hydrophyschidae; snail, family Lymnaeidae; dragonfly, family Gomphidae) along the Osor stream

		UP-1		UP-2		M		DM-1		DM-2	
		Conc.	BCF/BAF	Conc.	BCF/BAF	Conc.	BCF/BAF	Conc.	BCF/BAF	Conc.	BCF/BAF
Zn	Periphyton	107.57 ± 59.77	5.00 ± 3.81	2396.58 ± 1643.64	86.72 ± 57.41	16006.12 ± 1053.56	55.57 ± 1.03	8535.16 ± 49.45	47.84 ± 23.26	2506.93 ± 728.4	69.83 ± 52.44
	Stonefly	245.40	1.64	443.73	0.36	2453.83	0.16	2531.65	0.30	563.59	0.19
	Caddisfly	191.54	1.28	544.79	0.44	2170.90	0.14	1826.46	0.21	702.90	0.23
	Snail	120.59	0.80	212.98	0.17	-	-	-	-	508.00	0.17
	Dragonfly	146.42	0.98	224.21	0.18	1505.57	0.10	925.68	0.11	318.57	0.11
Mn	Periphyton	1682.59 ± 1152.5	74.40 ± 28.75	3540.9 ± 400.21	126.40 ± 28.87	11637.92 ± 988.44	92.68 ± 4.93	5628.91 ± 931.17	100.99 ± 22.82	3500.84 ± 1878.96	397.60 ± 335.78
	Stonefly	378.34	0.15	474.99	0.12	1301.91	0.12	1290.77	0.21	384.16	0.08
	Caddisfly	556.47	0.22	835.63	0.22	776.85	0.07	558.38	0.09	746.70	0.15
	Snail	136.62	0.05	142.57	0.04	-	-	-	-	155.68	0.03
	Dragonfly	193.75	0.08	540.66	0.14	1134.61	0.10	583.37	0.09	196.51	0.04
Fe	Periphyton	23548.52 ± 5263	221.85 ± 46.60	16370.69 ± 594.35	418.56 ± 108.31	31655.85 ± 7875.66	312.51 ± 15.71	16365.06 ± 2840.82	137.49 ± 1.36	24551.1 ± 5299.65	1597.71 ± 1397.75
	Stonefly	4302.53	0.16	3031.89	0.18	4950.73	0.19	2972.21	0.21	3930.78	0.14
	Caddisfly	2659.18	0.10	2252.62	0.13	4721.48	0.18	1755.29	0.12	2651.26	0.09
	Snail	1926.18	0.07	1629.62	0.10	-	-	-	-	3247.31	0.11
	Dragonfly	1291.84	0.05	865.46	0.05	2546.54	0.10	1012.56	0.07	2576.57	0.09
Pb	Periphyton	27.97 ± 17.57	<dl	177.69 ± 72.99	<dl	967.18 ± 160.15	<dl	342.72 ± 39.82	<dl	249.01 ± 83.77	<dl
	Stonefly	11.46	0.28	41.04	0.18	122.09	0.11	65.36	0.18	37.32	0.12
	Caddisfly	4.53	0.11	34.61	0.15	114.35	0.11	38.76	0.10	25.13	0.08
	Snail	2.04	0.05	10.81	0.05	-	-	-	-	9.92	0.03

	Dragonfly	1.94	0.05	19.92	0.09	65.35	0.06	24.85	0.07	19.15	0.06
Cr	Periphyton	9.45 ± 4.59	<dl	8.73 ± 0.13	<dl	11.93 ± 1.91	<dl	9.51 ± 2.88	<dl	12.44 ± 2.53	<dl
	Stonefly	2.05	0.16	1.12	0.13	1.47	0.14	1.99	0.27	1.88	0.13
	Caddisfly	1.30	0.10	1.14	0.13	0.71	0.07	0.52	0.07	0.75	0.05
	Snail	0.75	0.06	0.48	0.05	-	-	-	-	0.91	0.06
	Dragonfly	0.61	0.05	0.26	0.03	0.44	0.04	0.30	0.04	0.95	0.07
Ni	Periphyton	6.44 ± 3.51	5.68 ± 1.20	6.53 ± 0.33	13.33 ± 0.68	57.84 ± 1.88	20.19 ± 4.11	39.23 ± 3.32	26.70 ± 10.84	18.08 ± 5.49	37.43 ± 32.84
	Stonefly	2.78	0.31	7.02	1.04	9.72	0.16	8.59	0.23	3.87	0.18
	Caddisfly	0.53	0.06	1.06	0.16	8.02	0.14	30.29	0.82	3.90	0.18
	Snail	6.66	0.75	2.20	0.33	-	-	-	-	4.53	0.21
	Dragonfly	3.56	0.40	17.43	2.58	4.65	0.08	3.74	0.10	0.50	0.02
Cd	Periphyton	0.532	<dl	5.4 ± 0.09	<dl	16.47 ± 0.24	<dl	9.17 ± 3.42	<dl	4.87 ± 2.21	<dl
	Stonefly	0.208	0.390	1.337	0.245	3.277	0.201	2.939	0.254	0.737	0.11
	Caddisfly	0.143	0.268	1.088	0.199	2.602	0.160	1.526	0.132	0.476	0.07
	Snail	0.584	1.097	3.105	0.569	-	-	-	-	1.114	0.17
	Dragonfly	0.217	0.408	0.340	0.062	1.524	0.094	0.850	0.073	0.690	0.11
Cu	Periphyton	17.14 ± 10.34	9.59 ± 2.10	16.53 ± 0.66	9.94 ± 6.51	21.96 ± 1.9	19.30 ± 14.93	17.66 ± 1.45	14.65 ± 0.49	29.8 ± 5.27	64.65 ± 42.30
	Stonefly	22.85	0.93	12.55	0.74	15.20	0.74	9.93	0.53	14.98	0.45
	Caddisfly	15.14	0.62	11.09	0.65	8.89	0.43	14.04	0.75	13.99	0.42
	Snail	64.83	2.65	67.44	3.97	-	-	-	-	80.13	2.39
	Dragonfly	27.31	1.12	13.77	0.81	15.13	0.73	18.79	1.01	14.31	0.43

Trace metal concentrations in macroinvertebrates were analyzed once during the experiment. Snails of the family Lymnaidae were not found in M and DM-1. The bioconcentration factor in periphyton was not calculated when metal concentrations in water were below the detection limit (<dl). The data of periphyton are shown as average ± standard deviation (*N* = 2)



F I G U R E 4 Linear relationships of Zn (a), Pb (b) and Cd (c) concentrations between periphyton and four families of macroinvertebrates along the Osor stream. Snails of the family Lymnaeidae were not found at Mine (M) and Downstream 1 (DM-1). Linear functions are shown in Table S1. Note axes are on a log scale

Fish treatment effects

Although 3 barbels died at the end of the enclosure experiment in some reaches (2 fish in Upstream 2 and 1 fish in Mine), it is unlikely this small change in fish density affected our results. In total, 34 different macroinvertebrate taxa were found during the exclosure/enclosure mesocosm experiment, of which 12 were caddisflies (Trichoptera); 6 were dipterans (Diptera); 5 were mayflies (Ephemeroptera); 4 were dragonflies or damselflies (Odonata); 3 were snails; 2 were beetles (Coleoptera), and 1 was both stonefly (Plecoptera) and annelid (Oligochaete). Linear mixed models showed that variation in macroinvertebrate densities and periphyton biomass were mostly explained by 'Reach' and 'Treatment' effects (Table 3) because the effects of 'Time' and 'Replicate' were not significant for any of the models tested ($P > 0.05$). Fish predation was not affected by metal pollution levels (Figure 5a-f), i.e., the Reach × Treatment interaction term was not statistically significant (Table 3). Treatment effects on macroinvertebrates were statistically significant for Diptera (Figure 5d). However, several macroinvertebrate responses approached statistical significance ($P < 0.1$), such as EPT taxa (Figure 5a) and total macroinvertebrate density (Figure 6). We also found that densities of snails and dipterans were remarkably lower in Mine and Downstream 1 (Table 3; Fig. 5c-d), whereas total macroinvertebrate density was remarkably lower in Mine reach (Table 3; Figure 6). EPT taxa also showed lower densities in Mine reach (Figure 5a), although differences among reaches were marginally significant (Table 3). At the lower trophic level, periphyton variables (chlorophyll-*a* concentration and AFDW) showed a significant decrease in biomass in the presence of fish, regardless of metal pollution levels in the stream (Figure 5e-f). Moreover, chlorophyll-*a* concentration was significantly different among the reaches (Table 3), with Upstream 1 having a lower concentration of chlorophyll-*a* compared to downstream reaches (Figure 5e).

TABLE 3 Results of Linear Mixed Models: effects of Reach and Treatment on density of macroinvertebrates, chlorophyll-*a* concentration and ash-free dry weight (AFDW) biomass

	Reach			Treatment			Treatment × Reach			Explained variation	
	SS	F value	*P*	SS	F value	*P*	SS	F value	*P*	R^2m	R^2c
EPT	14.64	3.12	0.066	11.20	9.55	0.091	7.13	1.52	0.216	0.44	0.58
OCH	1.99	0.83	0.538	0.099	0.16	0.538	2.76	1.14	0.350	0.14	0.36
Diptera	6.06	4.86	**0.019**	5.78	18.52	**<0.001**	0.88	0.71	0.592	0.41	0.43
Snails	1.46	10.93	**0.001**	0.03	0.95	0.431	0.20	1.51	0.219	0.51	0.62
Total density	205.32	7.33	**0.005**	72.81	10.40	0.084	27.58	0.99	0.427	0.55	0.68
Chlorophyll-*a*	0.027	3.09	**0.024**	0.066	30.80	**0.031**	0.01	0.83	0.516	0.52	0.54
AFDW	0.24	1.73	0.221	3.55	103.61	**<0.001**	0.06	0.44	0.776	0.64	0.67

The degrees of freedom are 1 for Treatment, and 4 for Reach and the Reach × Treatment interaction term. Table shows variation explained by the fixed effects, i.e., 'Reach' and 'Treatment' (R^2m); and by the sum of the fixed effects and the random effects, i.e., 'Time' and 'Replicate' (R^2c). EPT = Ephemeroptera-Plecoptera-Trichoptera orders; OCH = Odonata-Coleoptera-Hemiptera orders. Note: No hemipterans were found during the experiment. Variables were transformed using Box-Cox transformation. Significant results ($P < 0.05$) are in boldface

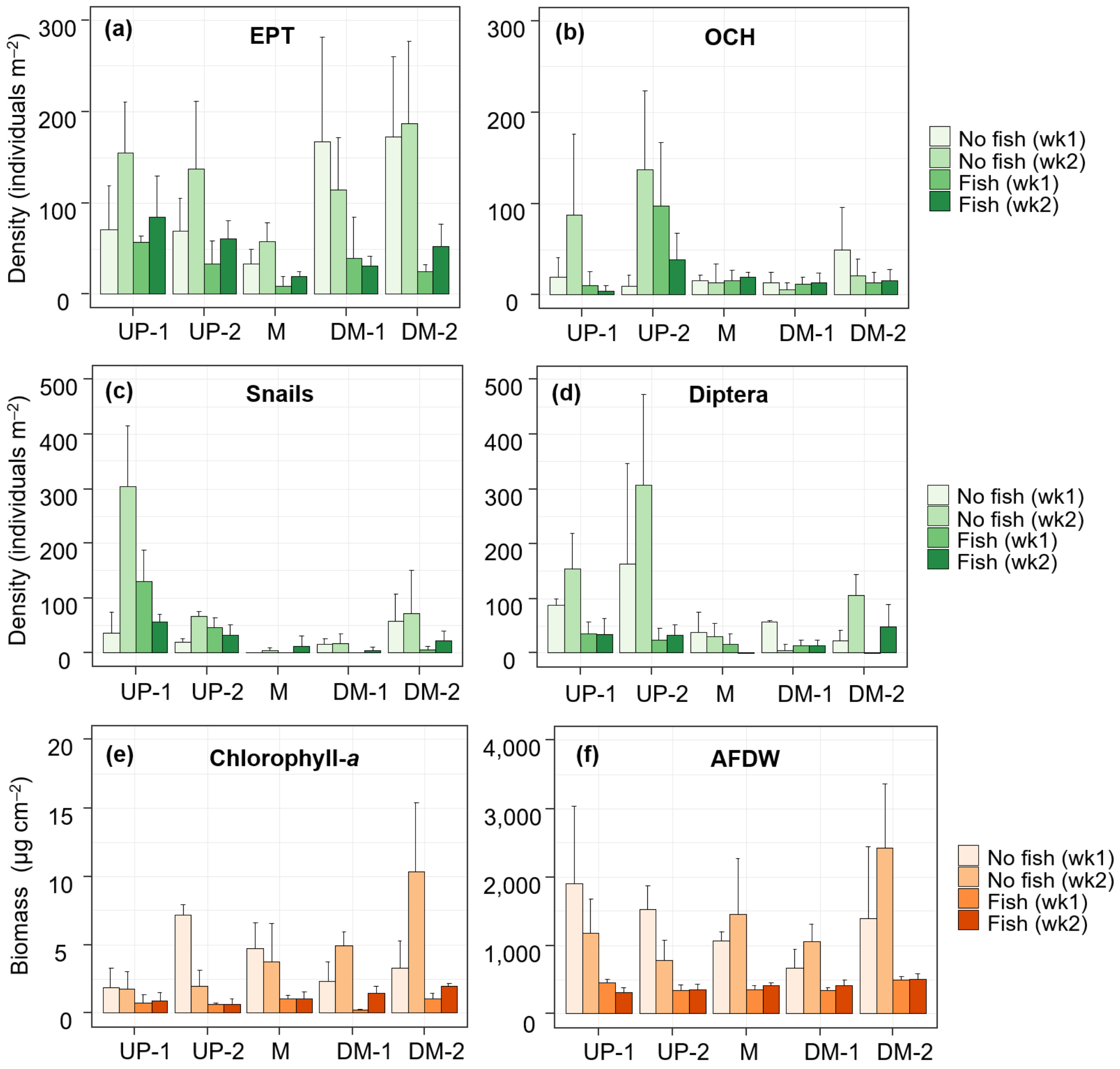
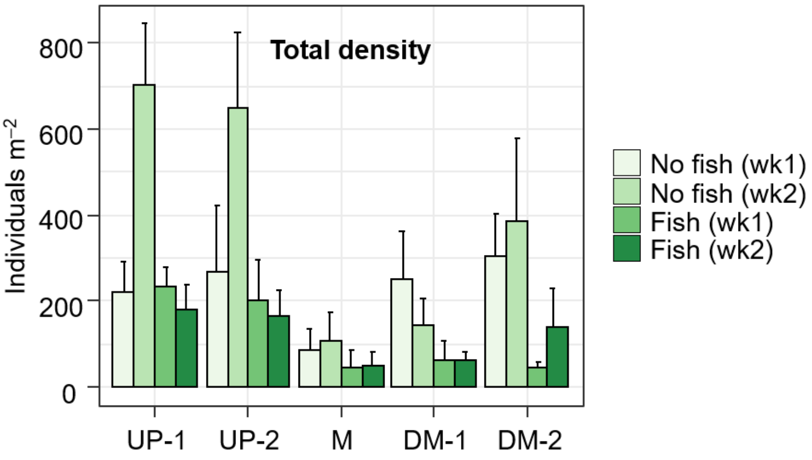


FIGURE 5 Density of benthic macroinvertebrates, and both chlorophyll-*a* concentration and ash-free dry weight (AFDW) biomass in response to the lack of fish ('No fish' treatment) and fish predation ('Fish' treatment). The two treatments had a duration of two weeks and surveys were conducted at day 7 (wk1) and 14 (wk2) over the course of each treatment period. Bar plots show mean ± standard deviation



F I G U R E 6 Total density of benthic macroinvertebrates in response to the lack of fish ('No fish' treatment) and fish predation ('Fish' treatment). The two treatments had a duration of two weeks and surveys were conducted at day 7 (wk1) and 14 (wk2) over the course of each treatment period. Bar plots show mean ± standard deviation

Discussion

The Osor stream showed a clear environmental stress gradient resulting from the interaction between metal pollution (direct metal inputs from the mine) and hydrological alteration (water diversion). As expected, the highest metal concentrations were found in reaches impacted by both environmental stressors (Tlili et al. 2011; Corcoll et al. 2012; Bonet et al. 2013, 2014; Argudo et al. 2020). We found Zn concentrations in water (maximum Zn value of 349 µg L^{-1}) that largely exceed the chronic toxicity threshold (120 µg Zn L^{-1}) for freshwater biota based on the criteria proposed by the U.S. Environmental Protection Agency (EPA, 2006). According to the 'biotic ligand model', which estimates bioavailability of metals based on hardness-based acute and/or chronic criterion (De Schamphelaere and Janssen 2004), the exposure to these Zn concentrations in slightly hard waters (15–50 mg Ca L^{-1}) would likely have harmful effects on stream communities (Argudo et al. in prep). In addition to Zn, we found high Mn concentrations in the stream waters of the Mine reach (143 µg Mn L^{-1}) and high Fe concentrations in all reaches (range = 47–132 µg Fe L^{-1}), but no toxicity to biota is expected according to the EPA's water quality criterion and previous research (Cadmus et al. 2018). Therefore, Zn was the primary chemical element driving the differences in toxicity observed among reaches (Atli et al. 2020).

Periphyton had the largest metal loads, but there was no evidence of biomagnification in the higher trophic levels. Indeed, metals rarely biomagnify in freshwaters (Farag et al. 1998; Goodyear and McNeill, 1999). Concentrations of Zn in periphyton increased gradually with dissolved concentrations in water, whereas this pattern was not observed for the other metals studied (Cr, Pb and Cd). These results suggest that, unlike other metals, Zn can be actively and rapidly adsorbed and/or taken up from water by periphyton (Kim et al. 2012). There were also significant relationships between the concentrations of various mining metals in periphyton and macroinvertebrates, as a reflection of the contaminant concentrations in water. Metal loads (Zn, Pb and Cd) differed among macroinvertebrate families of different feeding guilds, with lowest metal concentrations in predators (Gomphidae), consistent with previous studies (Farag et al. 1998; Goodyear and McNeill, 1999; Santoro et al. 2009). However, we acknowledge that the sampling size used for each family was small (only one sampling per site) which diminishes the predictive power of linear regressions. Moreover, we were unable to quantify metal concentrations in grazing snails, *Radix sp.*, (Lymnaeidae) along the entire metal pollution gradient because they were extirpated from the most polluted reaches.

Contrary to the first hypothesis, periphyton biomass did not decrease due to metal exposure, as previously reported by other studies (Hill et al. 2000; Morin et al. 2007; Bonet et al. 2013). According to the literature, several factors may explain the lack of a response in periphyton across the metal pollution gradient. First, shifts in abundance of dominant algal species as a result of differential susceptibility to metals (Sabater et al. 2002a) may complicate interpretation of routine biomonitoring studies (Medley and Clements, 1998). Second, the impacts of multiple-stressor occurrences on periphyton are uncertain (Calapez et al. 2020) and change temporally (Romero et al. 2019). In addition, the natural variability of abiotic and biotic factors along environmental and spatiotemporal gradients in streams (Clements et al. 2016) also adds another source of uncertainty in the prediction of stressor-effects relationships (Tlili et al. 2011). For instance, periphyton responses to metal exposure might be masked by longitudinal variation in trophic interactions (e.g. top-down control), as supported by the results of our mesocosm experiments. Like other studies have found (Clements and Rees, 1997; Clements et al. 2000; Courtney and Clements, 2002; Marqués et al. 2003; Solà et al. 2004), macroinvertebrate grazer density decreased with increasing metal pollution, possibly because Zn concentrations in water and bioaccumulated in periphyton were sufficiently elevated to cause toxicity to this group of invertebrates (Mebane et al. 2017). In addition to grazers, Clements et al. (2000) found strong effects of heavy metals on predators, and to a lesser degree on shredders and collectors. This variation in the degree of response of the functional feeding groups to metals might change among streams due to different sensitives of the corresponding species. We also found that detritivorous stoneflies of the family Leuctridae dominated macroinvertebrate communities at metal-polluted sites. This macroinvertebrate family has been found to be tolerant to environmental stressors in streams, such as pollution and acidification (Ledger and Hildrew, 2005; Layer et al. 2013).

Some predictions from classic food web theory (Carpenter et al. 1985) were not supported in the Osor stream, based on the invariant densities of some groups of invertebrates. The apparent lack of effects of predatory invertebrates (e.g. Odonata) on lower invertebrate trophic levels was likely a result of their overall low density (also seen in the descriptive study) and high variability in treatments. Even so, when predatory invertebrates are relatively abundant their impacts on ecosystems may not be ubiquitous (Kiffney, 1996; Clements, 1999). For instance, Rodríguez-Lozano et al. (2015) found that the absence of *Barbus meridionalis* increased both the abundances of mesopredators and their prey in an intermittent

Mediterranean stream, indicating that the top predator was more effective than predatory invertebrates at suppressing prey. In fact, larger predators are expected to generate greater impacts across multi-trophic levels than smaller predators (DeLong et al. 2015). Therefore, it appears that invertebrate grazers acted as the most important intermediators in this periphyton-based food web.

Results of the exclosure experiment agreed with the hypothesis that metal pollution can alter the outcome of species interactions. In the absence of fish (*Barbus meridionalis*), chlorophyll-*a* concentration (as a proxy for autotrophic community biomass) was significantly lower in the upper reach likely due to higher grazing pressure (Feminella and Hawkins, 1995), as reflected by the concomitant increase in density of snails and other taxa (e.g. mayflies). Conversely, this trophic cascade was not observed for the downstream reaches with different levels of pollution. Fleeger et al. (2003) reviewed indirect toxicant effects in aquatic ecosystems across of 150 studies, and pointed out that the abundance of primary producers (i.e. benthic micro- and macroalgae) can be commonly altered in streams by contaminant-induced changes in grazing rates. This means that grazers can be selectively eliminated through mortality or that the grazing behavior (trait changes) can change by direct toxicant effects (Clements and Rohr, 2009), thereby leading to indirect positive effects on periphytic algae. Our results are consistent with those findings, suggesting that periphyton communities might benefit from lower grazing pressure in metal-polluted streams lacking fish, and therefore consumer-resource interactions may play a central role in determining periphyton responses to water pollution (Calapez et al. 2020). In agreement with our results, other studies also reported changes in chlorophyll-*a* in response to predator removal, whereas ash-free dry weight remained invariant (e.g. Ludlam and Magoulick, 2010). Total periphyton biomass often has an important fraction of debris, which grazers may not feed on, so that variation in chlorophyll-*a* concentration is likely to be more linked to differences in grazing pressure.

Contrary to our expectations, trophic cascade strength did not vary as a function of environmental stress. Beyond the upper reach (Upstream 1), it would be expected that the absence of *Barbus meridionalis* leads to trophic cascades in the least polluted reaches (e.g. Upstream 2) given that grazer densities in those stream reaches were apparently similar. However, as mentioned above, trait-mediated interactions (e.g. changes in grazing rate or resource switching by herbivores; Schmitz et al. 2004; Clements and Rohr, 2009; Saaristo et al. 2018) may explain the lack of detectable trophic cascades in the metal-polluted sites. Furthermore, we cannot rule out that top predator removal led to species-level cascades as a result of minor interactions among species within a compartment of the food web. Even so, they do not usually explain habitat-wide changes in biomass distribution between trophic levels unlike community-level cascades (Polis et al. 2000). In addition, in line with the 'stress gradient hypothesis' (Menge and Sutherland, 1987), previous studies found that high environmental stress can decouple biotic interactions in aquatic ecosystems (Cheng and Grosholz, 2016). In a similar earlier study, Rubio-Gracia et al. (2017) found that the absence of *Barbus meridionalis* did not trigger trophic cascades involving post-flood invertebrate assemblages in streams impacted by water diversion and eutrophication, suggesting that a great deal of environmental stress may attenuate top-down effects. Thus, with the exemption of the upper reach, it is likely that downstream reaches showed greater environmental variation due to the combination of human-induced water stress (water diversion) and metal pollution, and consequently, the more unstable communities were unable to effectively control the higher standing crop of periphyton in fishless treatments.

Results of the enclosure experiment showed that fish predation effectively reduced the density of Diptera in cages, which is consistent with previous studies showing that Chironomids were the most abundant taxa in the gut contents of *Barbus meridionalis* (Mas-Martí et al. 2010; Rodríguez-Lozano et al. 2016b). However, this top predator has a generalized diet, also being primary consumers (e.g. mayflies) and other invertebrates frequent prey sources (Mas-Martí et al. 2010; Rodríguez-Lozano et al. 2016b). As with Diptera, we found that total macroinvertebrate density and the density of EPT taxa tended to decrease during the fish treatment, but these macroinvertebrate responses were not significant because of relatively high variation. Nevertheless, there is compelling evidence that the top-down control of this predatory fish can change whole macroinvertebrate composition and abundance (Rodríguez-Lozano et al. 2015; Rodríguez-Lozano et al. 2016a). Unfortunately, we cannot distinguish between consumption and prey turnover in cages. High immigration rates can overwhelm predation effects, while emigration can lower the risk of being eaten by the top predator (Clements, 1999). A predator avoidance behavior has been observed, for instance, in mayflies during predator-inclusion experiments (e.g. Tikkanen et al. 1994).

Interestingly, periphyton was predominantly controlled by *Barbus meridionalis* (up to 79% decrease in biomass at the end of the experiment), thus suppressing any potential cascading effects. Predatory fishes commonly affect periphyton through cascading trophic relationships (Dahl, 1998; Moulton et al. 2010; Winkelmann et al. 2014). The reduced periphyton in fish treatments can be attributed to two ecological processes associated with benthic foraging movements (Moore, 2006): 1) predatory fishes can accidentally ingest periphytic algae and detritus while foraging benthic invertebrates (Mas-Martí et al. 2010); and 2) bottom-feeding taxa can decrease standing stocks of periphyton through severe perturbation of substrates (Power 1990), the so-called ‘bioturbation effect’ (Fleeger et al. 2006). Our results indicate that *Barbus meridionalis* could act as ecosystem engineer in streams through the physical perturbation of benthic habitats (Jones et al. 1997), thereby controlling the food supply to invertebrate grazers and other aquatic animals. Furthermore, the presence of fish modified the indirect effects of metals on ecosystem structure, i.e., an increased periphyton biomass that would otherwise occur in the absence of fish. Identifying which organisms engineer habitats in varying environments provides a valuable knowledge for ecosystem conservation (Crain and Bertness, 2006), and therefore our results can have implications for the management of natural and human-altered ecosystems. Given the certainty that *Barbus meridionalis* can mediate ecosystem structure and functions (Rodríguez-Lozano et al. 2015; Rodríguez-Lozano et al. 2016a; Rubio-Gracia et al. 2017), we recommend that managers consider the importance of top trophic levels in decision-making processes to better predict management outcomes.

Conclusions

The present study is the first to have assessed the responses of benthic communities to the presence or absence of a predatory fish (*Barbus meridionalis*) in a stream with a gradient of metal pollution. Our results indicate that metal pollution can alter the outcomes of species interactions. Top predator removal led to cascading effects on periphyton biomass (chlorophyll-*a* concentration) in the upper (Reference) reach without metal pollution. On the contrary, along downstream reaches, direct negative (lethal and sublethal) effects of metals on macroinvertebrate grazers resulted in indirect positive effects on periphyton biomass (both chlorophyll-*a* and AFDW), thus reflecting metal-induced changes of biotic interactions. Moreover, the presence of the top predator led to decrease both macroinvertebrate density and periphyton biomass, irrespective of the degree of metal pollution. Thus, we found evidence that this top predator can strongly modify benthic habitats (i.e. periphytic algae attached to cobbles) through ecosystem-engineering impacts (bioturbation). Consequently, fish modified the above-mentioned indirect effects of metals on periphyton communities. Results of the present study are consistent with previous research (Rodríguez-Lozano et al. 2015; Rubio-Gracia et al. 2017) that highlighted the important functional role of *Barbus meridionalis* in natural and human-impacted streams. Together, these results suggest that the local extinction of top predators may exacerbate environmental stressor effects on ecosystem structure. Documenting the complexity of trophic relationships in varying environments provides a fundamental basis for the understanding of the effects of human impacts on fluvial ecosystems (Clements and Rohr 2009; Segner et al. 2014). We concluded that trophic-level-based ecosystem approaches should be considered in future ecotoxicological studies to improve predictions of population or community changes at contaminated sites.

5. GENERAL DISCUSSION

Mediterranean-climate rivers and streams are ecologically unique, with flow regimes reflecting precipitation patterns. Sequential flooding and drying, coupled with anthropogenic influences make these streams among the most stressed riverine habitat worldwide (Bonada and Resh, 2013). Human appropriation of water resources can significantly alter the variability of the natural flow regime, affecting the composition, abundance and diversity of non-adapted species, which can in turn lead to changes in ecosystem functions (Sabater et al. 2018). Moreover, hydrologic alterations can mediate the effects of other antropogenic stressors, such as water pollution and invasive species (Meißner et al. 2019). Current lines of research focus on assessing the effects of these anthropogenic stressors on stream communities, with the purpose of increasing mechanistic understanding of human impacts on fluvial ecosystems (Argudo, 2020). Nevertheless, one of the challenges to achieve this goal lies in the fact that organisms in ecosystems are interlinked by biotic interactions in ecological networks, so that actions of stressors at disparate sites within the network can lead, via indirect or cascading effects, to unexpected outcomes (Segner et al. 2014). Thus, research should take into account the network characteristics and biotic interactions into analyses of multiple-stressor effects on ecosystems (Bruder et al. 2019). In an attempt to address this challenge, this thesis has analized the effects of flow alterations, either by changes in water velocity or reductions in stream discharge, and of metal pollution on riverine fish species, invertebrate communities and periphyton, and their interactions. The main results highlighted that:

- There exists a large intra- and inter-specific variation in swimming capabilities (critical swimming speed, U_{crit}) and physiological traits (maximum metabolic rate, MMR; absolute aerobic scope, AAS) across diverse fish species. These findings suggested that not all fish can deal with flow variations in the same manner, and that physiology of an organism could constraint its capacity of resistance or resilience.

- The top predator (*B. meridionalis*) can mediate the effects of anthropogenic stressors on the stream food web. In situations where hydrologic alterations and the absence of the top predator co-occur, macroinvertebrate grazers benefitted from a lower fish predation, giving rise to increase the phosphate uptake efficiency of a reduced periphyton biomass. Conversely, metal contamination of stream waters caused the removal of macroinvertebrate grazers, leading to positive indirect effects of stressors on periphyton biomass in the absence of top predators.

5.1 Mechanisms underlying fish responses to flow variation

Our velocity tests were not meant to mimic the characteristics of a natural stream flow, but these tests do indicate relative physiological abilities of species to withstand high current velocities during flood events (Alexandre et al. 2014; Silva et al. 2021). Moreover, these experimental approaches to simulate flow variability conditions can provide valuable insight on the energetic costs associated with critical swimming performance (Beamish, 1978; Hammer, 1995). If fish face an increase in flow velocity, they will need to swim faster to maintain their position in the water column (Plaut, 2001), thus generating increased energetic costs (Webb, 1975; Videler, 1993). Considering the definition of energetic costs, i.e. the necessary energy to transport one body mass by one unit distance (Schmidt-Nielsen, 1972), it makes sense that increases in water velocities result in higher oxygen consumption rates (Chapters I & II; Ohlberger et al. 2005; Srean et al. 2017) and that these changes could represent a transient stress response (Costa et al. 2017). Thus, it is reasonable to think that not all fish will show the same water velocity tolerance given the enormous diversity of freshwater fishes. This thesis addressed how fish design features explain the variation in swimming performance and efficiency. Body mass was positively related to swimming performance because it is directly related to body volume and swimming thrust (Ohlberger et al. 2005; Srean et al. 2017).

Moreover, heavier fish swim more efficiently than their conspecifics with lower weight (Chapter II) because small-bodied fish have a larger surface in contact with water per unit of volume, which increases friction drag (Webb, 1975). This finding would therefore explain why smaller fish are more susceptible to be dragged downstream by flooding (Harvey, 1987). In addition, shallow caudal peduncle depths (Chapter I), fusiform bodies and higher proportion of muscle fibers (Chapters I & II) were identified as key morphological traits that enhance swimming capabilities of fishes, in agreement with previous studies (e.g. Webb, 1984; Scarnecchia, 1988; Fisher and Hogan, 2007; Ohlberger et al. 2016). Because there exists a close link between streamflow variability, swimming capacity and morphological characteristics in fishes (Levy and Bonner, 2009; Alexandre et al. 2014), it can be concluded that swimming performance tests are a useful tool to evaluate the potential effects of hydrological disturbances on riverine fish species.

The natural flow paradigm predicts that native species are more likely to benefit from free-flowing conditions, whereas low flows likely favor non-native species (Poff et al. 1997; Naiman et al. 2008). The reason is that native species are generally better adapted to lotic habitats with frequent occurrence of high-flow events (Minckley and Meffe, 1987), although the mechanisms by which invasive species appear to be disproportanely removed after flooding are not clearly understood. Given that swimming peformance can determine the capacity of an individual to withstand high current velocities (Alexandre et al. 2014), differences in swimming capability among species may explain the observed patterns of differential removal (Propst and Gido, 2004; Gido et al. 2013). However, our results do not support this hyphotesis, since we observed that some invasive species can swim faster (higher U_{crit}) than some native species of similar size. For instance, the invasive bleak (*A. alburnus*) exhibited higher swimming performance than some native species (Chapter II), such as the Catalan chub (*S. laietanus*) or the Mediterranean barbel (*B. meridionalis*). For its part, Cano-Barbacil et al.

(2020a) reported that invasive species exhibited swimming performance similar to native species, when accounting for a large set of freshwater fish species. Therefore, it seems that swimming capabilities alone would not explain the disproportionate removal of invasive fishes via flooding. Ward et al. (2003) proposed that behavioural responses to high flows could also influence the capacity of a species to persist in streams during flood events. Generally, bottom-dwelling fishes can use body and fin shape to increase downward friction and hold position (Adams et al. 1999). Thus, it is plausible to think that the pelagic behaviour of *A. alburnus* may be disadvantageous on holding position under high flows compared to the benthic behaviour of *B. meridionalis*. In fact, *B. meridionalis* prefers fast flowing riverine habitats (Merciai et al. 2018), whereas *A. alburnus* is mainly found in reservoirs (Vinyoles et al. 2007).

Differences in the timing of reproduction and of flooding (Harvey, 1987), life-history strategies (e.g. fecundity, age at maturity; Vila-Gispert et al. 2005), and physiological limitations and tolerances (Frimpong and Angermeier, 2010) can also influence riverine fish communities. Interestingly, our results showed that certain native species have higher absolute aerobic scope than invasive ones of similar size. This means that native species may have greater capacity to support energetically expensive processes aerobically, such as growth and reproduction (Guderley and Pörtner, 2010). Generally, individuals with higher metabolic rates are predicted to grow more quickly on average, mature earlier, and have more offspring per reproductive bout, although it depends on the species (e.g. White et al. 2016). In line with this hypothesis, Bernardo et al. (2003) suggested that native species may quickly recover (high recruitment of juveniles) following disturbance due to increased reproductive output, among other factors. Moreover, decrements in aerobic scope can compromise the capacity of animals to cope with fluctuating environmental changes, such as temperature (Pörtner and Knust, 2007), or other challenges (Bochdansky et al. 2005). Taken together, a combination of behavioural, morphological and physiological adaptations is most likely the mechanism that allows native fish to persist in streams that experience unpredictable abiotic perturbations.

5.2 Local environmental conditions shape the structural and functional responses of stream communities

Field monitoring studies of this thesis were conducted in the Llémena and Osor streams. These two watercourses have in common that are tributaries of the Ter River, but they differ from each other in hydrological and chemical (mineralization and pollutants) characteristics, watershed land use, etc. Broadly, the Osor stream has a much higher discharge than the Llémena stream (Table 1), and the Osor stream's stonebedded geological substratum is mainly siliceous, whereas that for the Llémena stream is calcareous and commonly precipitate calcium carbonate in the mineral form of calcite ($CaCO_3$). Moreover, while the lower part of the Llémena stream has a moderate nutrient enrichment due to the impacts of urban and agricultural land uses, the Osor stream is affected by the nutrient inputs from a wastewater treatment plant (WWTP) at St. Hilari Sacalm. Both streams showed orthophosphate (PO_4^{-3}) concentrations (as a proxy for the soluble reactive phosphorus, SRP) that largely exceeded the thresholds values ($0.025\ \mathrm{mg \cdot L^{-1}}$) supporting good ecological status for running waters (Phillips et al. 2018), although the PO_4^{-3} concentration was four times greater in the Osor stream (Table 1).

It is well-known that periphyton growth can be affected by abiotic characteristics of streams such as amount of shading from riparian vegetation and background nutrient concentrations, among other abiotic factors (Hillebrand and Kalhert, 2001). The more open canopy along the Llémena stream favours green algae (e.g. *Cladophora*) development because both light and nutrients do not limit algal growth (Guasch et al. 1995). In this stream both the ammonia and phosphate uptake rates were positive (Table 1), indicating an important contribution of periphyton to nutrient removal. In addition, it is important to note that the co-precipitation of phosphate with calcite contributes to decrease water nutrient concentrations, and potentially to attenuate the eutrophication response to phosphorus loading, as observed in other carbonate-rich ecosystems (e.g. Hamilton et al. 2009). In contrast, as occurs in mountain streams and headwaters, the extensive riparian vegetation along the Osor stream substantially reduces streambed light availability (Figure 1b) and hence limits primary producers (Bonet et al. 2013). Moreover, Argudo et al. (unpublished) found that periphyton assemblages from the Osor stream did not show a high nutrient uptake capacity, and the negative rates of nutrient uptake would indicate nutrient release into water (Table 1). Similarly, previous studies also found that the periphyton nutrient uptake efficiency can significantly decrease due to nutrient saturation in streams (e.g. Price and Carrick, 2016). Moreover, periphyton showed very high values of C:P ratio, indicating that the phosphorus content was low, but instead the C:N ratio was within the rage for the optimum algal growth (C:N < 10, according to Hillebrand and Sommer, 1999). Collectively, these structural and functional responses would therefore indicate that periphyton assemblages from the Osor stream are primarily nitrogen limited. Thus, the negative rates of ammonia (and nitrate) uptake by periphyton in some stream reaches suggest that periphyton assemblages preferentially take up nitrogen diffusing from the nitrogen-enrich substratum rather than nitrogen from the water column (von Schiller et al. 2007).

Beyond habitat characteristics, our predator removal experiments revealed that the top-down control by grazers was directly or indirectly responsible for the variation in primary producers (i.e. periphyton chlorophyll-*a* concentration and total organic biomass) in the streams studied (Figure 1). This finding is not suprising since previous studies also documented the importance of grazing pressure in controlling periphytic algal biomass (Feminella and Hawkins, 1995; Hillebrand and Kalhert, 2001; Hillebrand et al. 2002; Barbee, 2005). Moreover, the impacts of grazing pressure were not only limited to control primary producer biomass, but also regulated the availability of nutrients to algae (decreased C:N ratio; Table 1). Grazers can enhance periphyton nutrient content through the excretion of nutrients (Hillebrand and Kalhert, 2001; Evans-White and Lamberti, 2005), removal of senescent cells, or increased uptake of nutrients by the remaining cells; the two latter supported by our experimental results (Chapter III). In addition, shifts in periphyton biomass were also attributed to indirect effects of stressors mediated by biotic interactions and ecosystem engineering impacts (as discussed below; Figure 2).

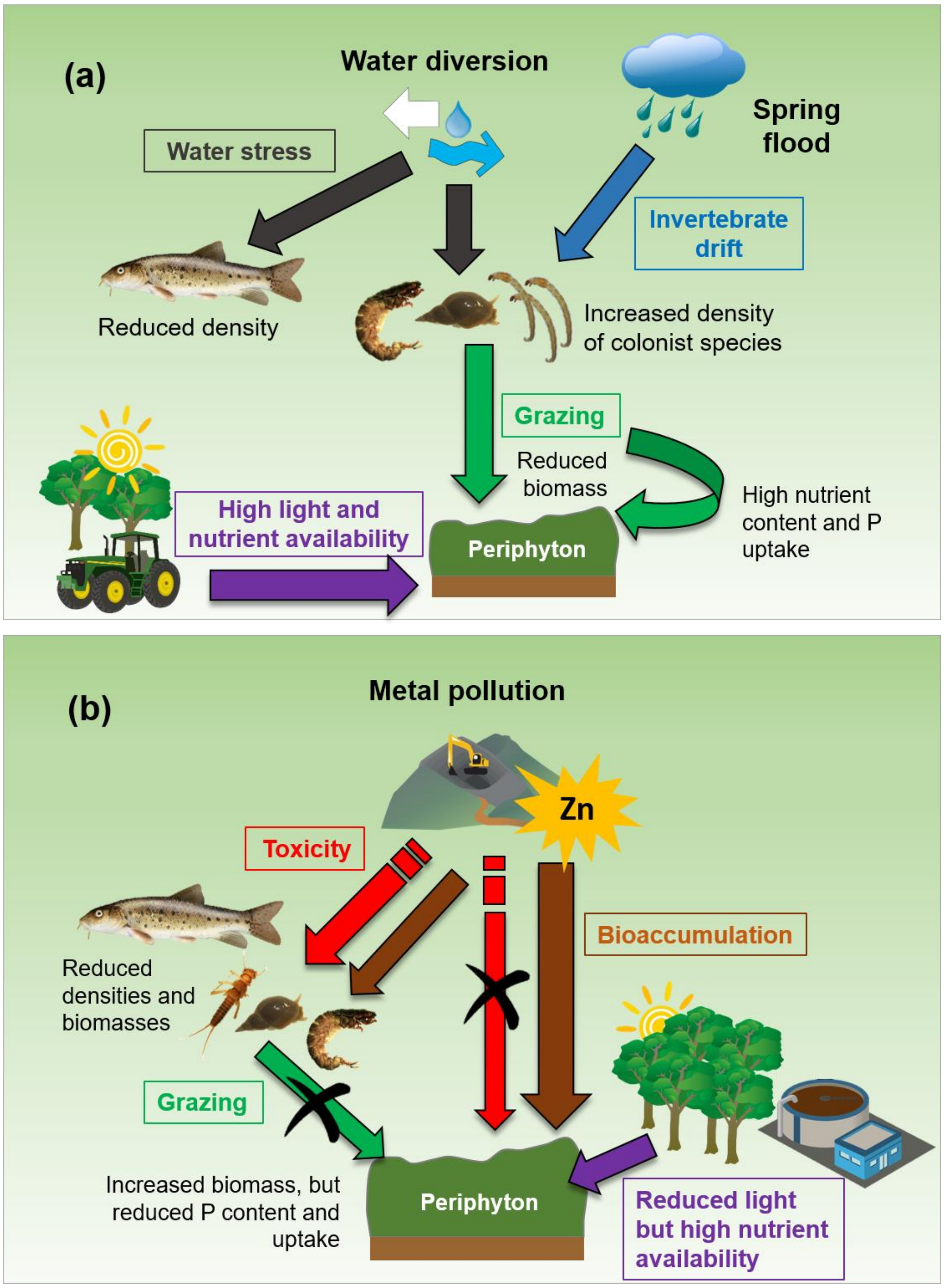
Our results also showed a direct cause-and-effect relationship between some natural and anthropogenic stressors and shifts in macroinvertebrate and fish assemblages. In the Llémena stream, most macroinvertebrate taxa were generalists (Diptera: Chironomidae) and classified as collector-gatherers (Table 1), indicating that flow regime alterations (continuos water diversion and spring floods) can strongly influence macroinvertebrate community structure. Many Chironomidae have asynchronous life cycles and high dispersal abilities that confer them a high resilience capacity following either flooding or drying (Fritz and Dodds, 2002, 2004). Moreover, the high abundance of Chironomidae can be directly associated with *Cladophora* density in regulated streams (Jansen et al. 2020). The higher macroinvertebrate density downstream could be explained by a rapid colonization of the regulated reach by invertebrates that has drifted from upstream perennial waters (Figure 1a), as reported elsewhere (Shivoga, 2001; Fritz and Dodds, 2004). In contrast, fish populations are expected to recover more slowly from hydrologic alterations due to their larger life-cycles and lower colonization rates (Allan and Flecker, 1993; Matthews and Marsh-Matthews, 2003). The barbel (*B. meridionalis*) density was strongly reduced downstream of the weirs in both streams (Table 1), with the sharpest decline found in the Llémena stream (up to 67% decrease in downstream waters; Table 1; Figure 1a). This native fish is highly sensitive to water diversion (Merciai et al. 2017), and the greater water depth of perennial reaches has been shown to substantially increase its survival probability and colonization rates (Merciai et al. 2018).

Metal pollution also affected benthic macroinvertebrate community structure, with shifts in total density and biomass (Table 1) and the removal of sensitive taxa. Similar to previous studies (Clements et al. 2000; Qu et al. 2010), the greatest effects were observed on mayflies (Ephemeroptera), which were highly sensitive and were reduced by 87 and 98% at Mine (M) and Downstream 1 (DM-1) reaches, respectively. Moreover, grazing snails of the genus *Radix* were extirpated from the most polluted reach (M), possibly due to the high metal concentrations accumulated in periphyton (16,000 µg Zn g DW^{-1}; Figure 1b). Consequently, the density of grazers also decreased with increasing Zn pollution (Table 1), as observed in previous studies (Courtney and Clements, 2002; Marqués et al. 2003; Solà et al. 2004). Similarly, barbel density also decreased in the most polluted reach (M) of the Osor stream (Table 1; Figure 1b). The recent study of Atli et al. (2020) investigated the physiological mechanisms (antioxidant capacity) of individuals of *B. meridionalis* (collected from the Osor stream) to counter metal exposure, and the authors concluded that common Zn concentrations in water (from 200 µg Zn L^{-1}) cause toxicity and produce deleterious effects on their physiological status. Collectively, all these results agree with the 'biotic ligand model' proposed by De Schamphelaere and Janssen (2004), in which concentrations of Zn between 300 and 400 µg L^{-1} (349 µg L^{-1} in the most polluted site; see Table 1) in slightly hard waters (15–50 mg Ca L^{-1}) are indicative of potential toxicity impacts, resulting in a decreased fish abundance by more than 10% (LC_{10} = 91–324 µg Zn L^{-1}) after chronic exposure.

TABLE 1 Mean values (± standard deviation) of the environmental and biological variables of the sampling reaches along the Llémena and Osor streams. Significant differences (in bold) in periphyton biomass and nutrient content, and total biomass and density of macroinvertebrates among reaches of the same stream are represented by different lowercase letters. Barbel density was measured once during the experiment carried out in the Osor stream

	Llémena stream		Osor stream				
	Permanent reach	Regulated reach	UP- 1	UP-2	M	DM-1	DM-2
Water							
Discharge ($m^3 \cdot h^{-1}$)	62.3(20.3)	42.4(42.5)	432(72)	180(36)	288(108)	216(36)	324(36)
Temperature (°C)	12.2(0.5)	12.9(0.8)	18.9(1.5)	20.1(1.6)	21.0(1.7)	20.0(1.2)	20.8(1.0)
Oxygen ($mg \cdot L^{-1}$)	11.1(0.7)	11.2(0.2)	8.4(0.5)	7.8(0.3)	8.1(0.2)	8.2(0.1)	8.2(0.3)
pH	8.4(0.3)	8.5(<0.1)	8.3(0.1)	8.1(0.1)	8.2(0.1)	8.2(0.2)	7.9(0.1)
Cond ($\mu S \cdot cm^{-1}$)	464	517	373(41)	416(82)	517(25)	484(18)	463(30)
PO_3 ($mg \cdot L^{-1}$)	0.3(<0.1)	0.3(<0.1)	1.5(0.3)	1.2(0.1)	0.9(0.1)	1.1(0.3)	1.3(0.0.3)
NH_4 ($\mu g \cdot L^{-1}$)	19.4(8.6)	36.3(18.6)	27.4(17.0)	33.5(14.2)	48.6(44.2)	25.5(6.9)	28.2(3.9)
NO_3 ($mg \cdot L^{-1}$)	2.1(0.3)	3.0(1.5)	2.6(0.7)	1.2(0.3)	1.0(0.4)	1.4(0.6)	1.5(0.5)
Zn ($\mu g \cdot L^{-1}$)	-	-	21.0(20.1)	25.4(10.9)	349.0(121.7)	225.0(93.5)	84.6(36.8)
Mn ($\mu g \cdot L^{-1}$)	-	-	20.8(7.8)	25.3(8.1)	143.4(34.8)	48.4(14.2)	24.7(10.8)
Fe ($\mu g \cdot L^{-1}$)	-	-	132.9(60.9)	128.2(152.2)	96.4(23.5)	91.7(40.0)	47.2(36.1)
Ni ($\mu g \cdot L^{-1}$)	-	-	0.8(0.7)	0.4(0.1)	3.3(1.1)	2.2(1.3)	1.6(1.0)
Periphyton							
Chlorophyll-*a* ($mg \cdot m^{-2}$)	**21.7(11.05)b**	**5.8(2.3)a**	10.8(8.1)	33.6(27.3)	27.0(22.6)	72.1(70.6)	70.2(80)
AFDW ($g \cdot m^{-2}$)	**10.4(6.8)b**	**1.2(1.1)a**	8.4(6.2)	9.8(2.6)	5.8(1.2)	10.4(5.8)	9.7(5.3)
C:N ratio	**18.6(0.41)b**	**10.9(4.25)a**	9.9(1.7)	9.1(1.1)	9.9(0.7)	8.9(0.8)	8.8(1.1)
C:P ratio	263.3(53.3)	129.4(107.7)	1155.6(512.9)	1745.6(1162.6)	1243.7(457.5)	1570.1(532.9)	1464.5(903.5)
Uptake P-PO_4^{3-} ($\mu mol \cdot m^{-2} \cdot min^{-1}$)	2.4(0.9)	1.8(1.6)	0.1(1.7)	-1.1(4.6)	-2.2(2.4)	0.4(2.3)	0.8(1.8)
Uptake N-NH_4^+ ($\mu mol \cdot m^{-2} \cdot min^{-1}$)	3.6(0.32)	7.3(7.1)	5.5(55.4)	1.1(4.4)	-4.4(8.9)	-0.6(6.1)	-3.3(4.4)

Macroinvertebrates							
Dry mass (g·m^{-2})	**0.1(0.0)a**	**0.4(0.2)b**	**0.4(0.1)ab**	**0.7(0.1)b**	**0.2(0.1)a**	**0.3(0.1)a**	**0.4(0.1)ab**
Total density (ind·m^{-2})	**321.8(169.5)a**	**1441.4(1094.2)b**	**1000.1(191.6)ab**	**1147.1(267.2)b**	**520.1(63.1)a**	**580.7(99.2)a**	**835.1(188.3)ab**
EPT %	13.4(9.3)	30.6(34.2)	61.6(13.6)	56.4(11.1)	63.6(3.4)	59.7(13.9)	59.9(17.0)
OCH %	0.4(0.4)	0.4(0.4)	23.4(9.1)	24.6(8.3)	16.5(5.2)	8.5(2.9)	21.2(10.8)
Diptera %	85.2(10.6)	68.8(34.1)	11.4(6.8)	15.8(5.5)	19.2(8.4)	30.3(10.6)	14.8(4.3)
Snails %	0.2(0.4)	0.2(0.3)	2.7(2.4)	2.8(2.9)	0.2(0.3)	1.5(1.3)	2.7(2.3)
Grazers %	4.7(4.2)	19.6(22.1)	29.1(3.1)	24.8(10.9)	10.1(2.1)	4.5(0.9)	22.1(9.5)
Shredders %	26.6(1.1)	22.1(6.6)	22.1(8.5)	26.1(5.5)	21.9(9.5)	27.4(8.3)	22.1(4.1)
Collector-gatherers %	65.5(6.5)	38.9(29.4)	26.2(7.9)	32.6(9.1)	30.3(10.3)	33.5(4.6)	27.2(5.2)
Collector-filterers %	0.8(1.2)	18.2(22.8)	14.6	9.4	27.4	21.5	18.8
Predators %	2.3(1.0)	1.2(0.5)	7.9(3.5)	6.9(2.2)	10.4(3.6)	13.1(2.4)	9.6(1.4)
Barbus meridionalis							
Total density (ind·ha^{-1})	6447(494)	2100(840)	2164	1986	1362	2419	4115



F I G U R E 1 Summary of the effects of natural and anthropogenic stressors, i.e. flow regime alterations due to water diversion and flood disturbance (a), and chemical pollution due to mine drainage and runoffs (b), and habitat characteristics (light availability depending on riparian forest development and nutrient enrichment due to agricultural activities and the presence of a wastewater treatment plant) on ecosystem structure (densities and biomasses, nutrient content) and function (periphyton nutrient uptake)

5.3 Perspectives on context-dependency of biotic interactions

The different results obtained from the mesocosm experiments indicate that the importance of top-down effects in structuring food webs can vary within stream ecosystems due to the differences in environmental conditions (abiotic factors) prevailing at the reach. Additionally, we observed differences in top-down processes between stream ecosystems, in part because experiments were carried out at different seasons. It is well-known that biotic communities of Mediterranean streams can change over time due to variations in habitats conditions (Gasith and Resh, 1999), so variations in the strength of biotic interactions might also occur (Beck et al. 2019). The post-flood invertebrate assemblages of Llémena stream in spring contrasts with the macroinvertebrate community of the upper reach (UP-1) of Osor stream in summer, with a larger proportion of other primary consumers (e.g. EPT taxa) and functional feeding groups beyond collector-gatherers (Table 1). While in this latter reach fish exclusion resulted in density-mediated indirect interactions (Figure 2a), i.e. decreased periphyton biomass due to increased grazing pressure (e.g. Flecker and Townsend, 1994), this trophic cascade was less evident in the Llémena stream. Similarly, we found that the density of Diptera significantly decreased in fish treatments, in agreement with previous studies showing that this taxon dominated barbel gut contents (Mas-Martí et al. 2010; Rodríguez-Lozano et al. 2016b), but contrasts with the weaker fish predation effects in the Llémena stream despite the higher availability of prey. Recently, Jellyman and McIntosh (2020) found that top-down processes declined with increasing flood disturbance, partly because flooding caused great variation in the dynamics of macroinvertebrate community structure (i.e. increased invertebrates without morphological defences), thereby affecting the strength of cascading predatory effects. Our results are in line with those of Jellyman and McIntosh (2020), and suggest that high production rates of primary consumers as a result of flow-related disturbances overwhelmed predation rates. Moreover, other factors such as increased flow velocity or nutrient enrichment can also mitigate top-down effects through the enhancement of both primary and secondary production (Blanchet et al. 2008; Davis et al. 2010). It therefore demonstrates that the bottom-up influence of disturbances on consumer community structure determined the strength of top-down control and consequently the food-web structure of streams.

Top-down effects were not affected by Zn contamination and the predatory fish (*B. meridionalis*) effectively controlled the density of dipterans (Diptera) and to a lesser degree the density of other invertebrate taxa (e.g. EPT) via consumption, but also the periphyton biomass via bioturbation. Consequently, fish suppressed potential trophic cascades by affecting different trophic levels in the food chain (Figure 2a-b). It seems that the benthic behaviour of this cyprinid fish can strongly alter standing crops of attached algae on cobbles, as also observed in other fish species (Power, 1990). Nevertheless, fish-mediated top-down control of producer biomass was not ubiquitous in all stream ecosystems (Figure 2). So the question arises, why are the effects of fish bioturbation larger in some streams than in others? This context dependency could be explained by: (i) changes in species composition of the periphyton with different morphological features (height, length and straightness of filaments) associated with physicochemical characteristics of streams (in calcareous streams the periphyton tends to be made up of encrusting communities; e.g. Guasch et al. 1995) that drive differences in vulnerability to drag friction (Reiter et al. 1986); (ii) by differences in the type of abiotic substrates and sediments among streams, which in turn influence the role of benthic 'bioturbators' (Jones et al. 1997); (iii) or by the influence of abiotic forces (e.g. hydrologic disturbances) diminishing the importance of engineering, or at least make it more difficult to detect (Vanni, 2010), as previously mentioned.

Studies focusing on trophic interactions in multi-stressed ecosystems have revealed indirect (positive or negative) stressor effects mediated by biotic interactions (Alexander et al. 2013; Rodrigues et al. 2018; Bruder et al. 2019). Two indirect effects of multiple stressors involving grazer-periphyton interactions were identified in our fishless treatments. In fact, species loss or the elimination of an entire trophic level may represent a biological disturbance (e.g. grazing pressure; Calapez et al. 2020) for many aquatic organisms. On the one hand, increased periphyton biomass associated with the presence of toxic chemicals (e.g. metals) are usually the result of density- and trait-mediated effects due to reductions on grazers abundance or of their feeding rates (Fleeger et al. 2003; Rodrigues et al. 2018), in accordance with our results (Figure 2b). On the other hand, fish exclusion may indirectly enhance periphyton phosphate uptake by increasing grazing pressure (Figure 2c), which favours an early successional community that requires high availability of resources (Haglund and Hillebrand, 2005; Proia et al. 2017). In contrast, other authors suggested that grazers can reduce the nutrient uptake capacity due to reduced demand of a lower periphyton biomass (e.g. Sabater et al. 2002b, and references therein). In other cases, however, stressor-induced changes in grazer density do not always lead to changes in periphyton functions. For instance, the thesis of Argudo (2020) showed that the capacity of periphyton to nutrient removal did not vary in response to fish exclusion and metal exposure (maybe due to compensatory mechanisms, e.g. Vinebrooke et al. 2004; Ghedini et al. 2015), in spite of the marked differences in grazer density along the Osor stream (Figure 2a-b). These contrasting outcomes certainly reveal the complexity of biotic interactions (i.e. nonlinear responses) that constrains our ability to forecast the ecological consequences of multiple stressor occurrences in fluvial ecosystems (Segner et al. 2014). As supported by our results, such projections are challenging because the effects of anthropogenic stressors may be exacerbated, reduced or even reversed by other environmental stressors or interactions among species.

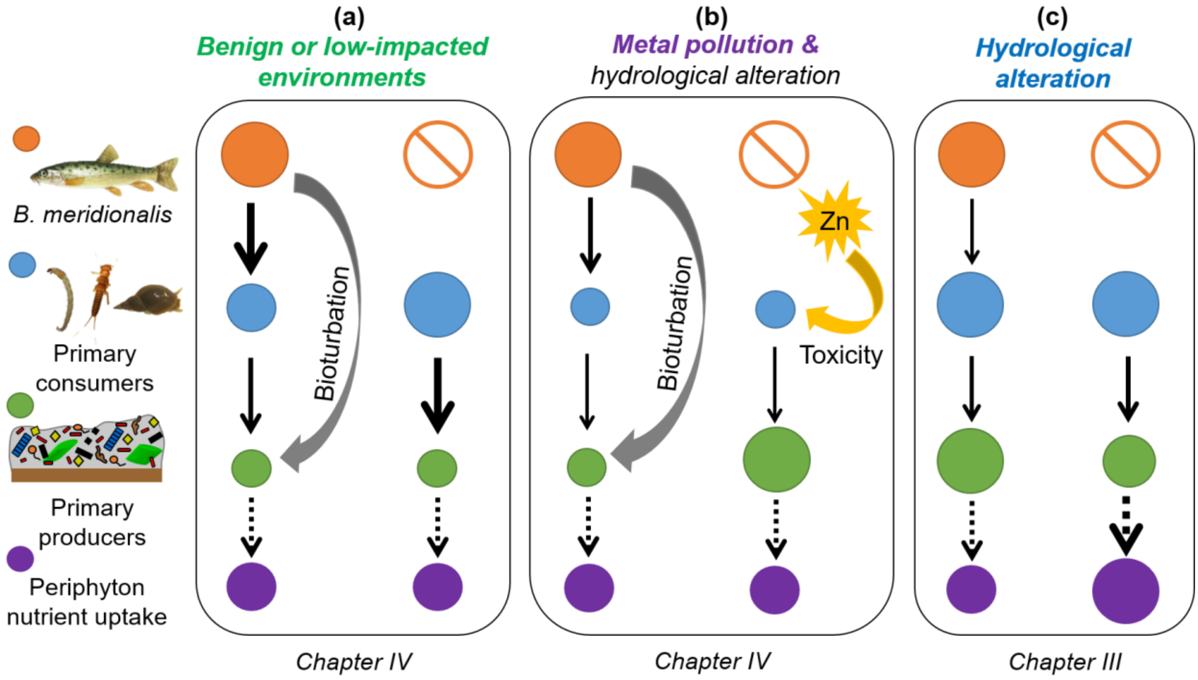


FIGURE 2 Diagram of the context-dependency of top-down effects of a predatory fish (*Barbus meridionalis*) on lower trophic levels (macroinvertebrate density – periphyton biomass and nutrient uptake). Darker solid arrows represent trophic interactions (predation or grazing), grey solid arrows represent fish bioturbation, and dashed arrows represent changes in periphyton functions (nutrient uptake rates) because of species interactions. The thickness of the arrow is proportional to interaction strength and the circumference size indicates the ecological outcome

5.4 Implications, management and future directions

This thesis provided data about swimming performance of several freshwater fish species, some of which had never been tested before, thus diversifying the existing information regarding the swimming capabilities of the Iberian ichthyofauna. The reported estimations of swimming speed can be used in future studies to assess fish abilities to cope with flow alterations (water flow tolerance), categorize their habitat preferences, or improve the non-native species management (Cano-Barbacil et al. 2020a), since a species swimming performance might be a limiting factor of its presence in a given habitat. In addition, given that slow flowing waters can represent the optimum flow conditions for invasive species, management actions focused on the recovery of natural flow regimes might help to control populations of invasive species, such as the pumpkinseed (*L. gibbosus*), and to decrease the pressure on the native species (Bernardo et al. 2003). Alternatively, deliberate high-flow releases from dams during the reproductive season may reduce the recruitment strength by disrupting spawning and by damaging and flushing away eggs and/or individuals (Harvey, 1987; Erman et al. 1988).

It is increasingly recognized that swimming speed is also a major determinant of passage success and migration rates (Haro et al. 2004; Tudorache et al. 2008; Jones et al. 2020). Thus, a thorough understanding of fish swimming capabilities can be crucial for the design of effective fish passes to facilitate passage over vertical barriers such as dams and weirs (Katopodis, 1992; Katopodis et al. 2019). In line with a recent study (Jones et al. 2020), the inter- and intra-specific variation in critical swimming speed was strongly influenced by body size (e.g. 26% CV in U_{crit} for *B. meridionalis*), but substantial interspecific variation was also observed even among species of similar body size, thus indicating that ‘one size fits all’ approach for estimating fish barrier passability is not always suitable. Therefore, it is important to account for the full range of swimming abilities that species exhibit in order to effectively mitigate size-related effects of barriers (Keefer et al. 2009; Katopodis et al. 2019), as well as identifying the drivers of variation in swimming speed beyond body size. Among others, morphological characteristics of species (Jones et al. 2020), phenotypic plasticity, or changes in willingness behaviour between different seasons (Silva et al. 2021) are also important factors that we need to take into account in order to improve barrier passability. Moreover, as energetic expenditure (measured as metabolic rates) increases rapidly with flow velocity in most of species (Chapter II), excessive energetic demanding passes due to an inadequate design may compromise the energy reserves of fish for reproduction and other life history functions (Thiem et al. 2016). Integrative studies of both swimming performance and metabolism can be thus of fundamental importance to understand why fish pass design may potentially fail, so as to improve passage efficiency (Noonan et al. 2012).

Respirometry experiments are becoming invaluable tools for conducting quick testing of many species to determine those physiological traits that enhance resistance and resilience to environmental pressures (Stoffels, 2015). Despite the weak swimming capability (i.e. reduced resilience capacity), *A. iberus* displayed higher absolute aerobic scope than *G. holbrooki* (Chapter I), which is in essence, the total aerobic capacity for somatic or gonadal growth, digestion and homoeostatic responses to environmental stressors (Clark et al. 2013). Therefore, for future studies, it would be interesting to demonstrate whether the increased aerobic scope of the native species can result in higher resistance capacity to survive in physiologically harsh environments, as changes in water temperature and salinity are known to mediate the competitive interactions between these sympatric species (Alcaraz et al. 2008a).

Given that future climate change scenarios in Mediterranean regions predict an exacerbation of drought conditions due to the rising temperature and declining annual precipitation, as well as an increase of the occurrence of extreme events (IPCC, 2007), it is plausible to think that some regulated streams will shift from permanent to intermittent or even ephemeral if the intense use of water resources persists (Larned et al. 2010; Filipe et al. 2013). Therefore, research on the effects of flow regulation on food-web structure and ecosystem functioning are becoming essential to forecast water-scarcity scenarios (Ruhí et al. 2016; Sabater et al. 2018). In particular, future investigations might be focused on assessing the capacity of benthic communities from intermittent reaches to cope with natural and anthropogenic disturbances, considering that Mediterranean river species, which have acquired traits to cope with the highly seasonal and predictable habitat connectivity losses (Bonada and Resh, 2013), might not be adapted to deal with additional environmental disturbances (Chapter III). The resulting knowledge can be pivotal to increase public awareness on the effects of human activity in the framework of the global change (Vargas-Amelin and Pindado, 2014).

The use of predator removal experiments has become an invaluable tool for untangling the relative importance of bottom-up and top-down forces that regulate the periphyton biomass and composition within and across ecosystem types (e.g. Gruner et al. 2008). The critical link in understanding the variation in periphyton biomass can be the trophic interaction between herbivores (grazers) and primary producers, as has been demonstrated throughout this thesis. However, other functional feeding groups (shredder, collector-gatherer) can play an important role in leaf breakdown processes (Rodríguez-Lozano et al. 2016a), thereby affecting periphyton microbial communities indirectly. Moreover, it is important to take into account that Mediterranean-climate streams and rivers are intrinsically variable, and the composition of benthic species (Vannote et al. 1980; Gasith and Resh, 1999; Bonada and Resh, 2003) and the strength of biotic interactions (Beck et al. 2019) shifts in space and time according to physical conditions (discharge, temperature, nutrient and light availability). At the same time, anthropogenic stressors can also influence the magnitude of biotic interactions (Chapter IV), and recent empirical evidence suggests that the impacts of environmental stressors can also vary over time (Romero et al. 2019). Therefore, continuing studies should document the spatio-temporal sequences of biotic interactions in order to have a full picture of the ecological consequences of anthropogenic stressors in fluvial ecosystems.

Effect-based methods for diagnosis and monitoring of water quality are recognised as an increasingly important tool for eco-toxicological risk assessments (Wenersson et al. 2015; Van den Brink et al. 2016; Brack et al. 2019). The reason is that traditional toxicological approaches, i.e. using toxicity endpoints that can be measured in laboratory test, ignore the additional physical and biological stressors that organisms encounter in their natural environment. In contrast, in situ experiments integrate the combined effects of complex environmental conditions and potentially variable exposure to toxic chemicals, thus becoming a more reliable and ecologically meaningful method for the assessment of toxicity (Connon et al. 2012). This thesis exemplified how field mesocosm experiments can provide insight into cause-effect relationships between anthropogenic stressors and biological responses, but also the environmental consequences associated with the presence or absence of fish, thus allowing us to predict future scenarios. In stream ecosystems where communities can be defined by the strength of species interactions (Chapter IV), multitrophic approaches can be thus of fundamental importance to identify direct and indirect effects of toxicants, and species that are crucial for transmitting stressor effects across trophic levels (Clements and Rohr, 2009; Segner et al. 2014; Bruder et al. 2019). In addition, selection of ecological indicators and the appropriate level of taxonomic resolution are also critical decisions when designing stream biomonitoring studies (Clements et al. 2000). Our results highlight the need to account for non-taxonomic categories, such as functional feeding groups and other functional measurements (e.g. periphyton nutrient uptake) into ecological studies. Moreover, functional responses can be more detectable than structural ones in relatively short-term experiments (Chapter III) and, in particular, variation in nutrient uptake capacity due to contaminant exposure can be an indication of eco-toxicological risk in running waters (Guasch et al. 2016; Proia et al. 2017). Thus, it follows that ecotoxicologists should also considerer functional attributes (what they do rather than who they are) when predicting responses to environmental stressors.

This thesis has demonstrated the important functional role that native fish species, such as the Mediterranean barbel (*B. meridionalis*), can play in human-impacted fluvial ecosystems. Given that the current biodiversity loss is biased towards species in the higher trophic levels (Schneider and Brose, 2013), understanding the relationship between top predators and ecosystem structure and functions is of primary importance. Generally, top predators cannot be included in small-scale experiments in many ecosystems, which has been the primary criticism of mesocosm experiments (Schindler, 1998). However, top predators in Mediterranean streams tend to have relatively small body sizes (Rodríguez-Lozano et al. 2015; 2016a), which makes easier their handle and use in caging experiments. In addition, protecting these species (especially ecosystem engineers) is certainly essential for preventing ecosystem services (Crain and Bertness, 2006). In ecosystems where the Mediterranean barbel is endangered reintroduction programs can be an effective tool for habitat restoration (Rodríguez-Lozano et al. 2015). Nevertheless, a close cooperation between investigators and managers in planning and decision-making processes is highly recommended due to the context-dependent effects of fish density on structuring benthic communities and modifying ecosystem functions.

6. CONCLUSIONS

CHAPTER I: Differences in swimming performance and energetic costs between an endangered native toothcarp (*Aphanius iberus*) and an invasive mosquitofish (*Gambusia holbrooki*).

1. Both sexes of *Aphanius iberus* showed similar swimming performance and metabolic traits, whereas males of *Gambusia holbrooki* performed better than females.
2. *Aphanius iberus* showed lower critical swimming speed, and higher maximal metabolic rate and absolute aerobic scope than *Gambusia holbrooki*, after controlling for body size.
3. *Gambusia holbrooki* showed lower minimum cost of transport and higher optimal swimming speed than *Aphanius iberus* for a given body mass. *Gambusia holbrooki* also showed lower mass-specific cost of transport at a comparable workload.
4. Caudal peduncle depth factor and fineness ratio significantly drive the differences in swimming performance and efficiency between the two species. The more hydrodynamic and streamlined body shape of *G. holbrooki* resulted in greater capacity to withstand the water flow and to reduce swimming costs.
5. It seems that the native toothcarp may be at a competitive disadvantage over the invasive mosquitofish wherever they coexist, especially for those ecological processes in which swimming is necessary, such as food capture and predator avoidance.

CHAPTER II: Size-related effects and the influence of metabolic traits and morphology on swimming performance in fish.

1. Body mass was largely the best predictor of swimming speed and metabolic traits within species. After accounting for body mass, critical swimming speed was independently affected by total length or maximal metabolic rate in certain fish species, indicating that the sign of the relationships between swimming capacity and other traits may not always be positive within species.
2. Standard metabolic rate and relative critical swimming speed were positively related to maximal metabolic rate. Maximal metabolic rate and relative critical swimming speed were also positively related to muscle and fineness ratios.
3. Among the study species, *Alburnus alburnus* performed best in terms of swimming performance and efficiency. However, *Lepomis gibbosus* attained lower mass-specific cost of transport than some streamlined species, possibly reflecting a cost reduction strategy to compensate for hydrodynamic disadvantages.
4. This study provided insight into the key factors influencing the swimming performance of fish at both intra- and interspecific levels.

CHAPTER III: Combined effects of hydrologic alteration and cyprinid fish in mediating biogeochemical processes in a Mediterranean stream.

1. In spring, local environmental conditions were critical for macroinvertebrate assemblages. The high abundance of chironomid larvae was attributed to the high resilience capacity of this taxon to cope with fluctuating changes in hydrological regime.
2. The variation in periphyton chlorophyll-*a* concentration and total biomass was inversely associated with the variation in macroinvertebrate density along the stream, thus reflecting a strong top-down control of grazers on primary producers.
3. Fish density treatments did not cause structural changes in stream benthic communities. Nonetheless, fish exclusion led to enhance periphyton phosphate uptake in the regulated reach, likely due to the effects of grazing. The lack of variation in nutrient removal by periphyton communities from the permanent reach suggests that hydrologic alterations can hamper the ability of stream biotic communities to compensate for the absence of fish.
4. It can be concluded that the density of fish (*Barbus meridionalis*) can mediate the effects of anthropogenic alterations on benthic communities and associated biogeochemical processes in flow-regulated sites.

CHAPTER IV: Indirect food-web interactions mediated by metal pollution and an ecosystem engineer, *Barbus meridionalis* (Cyprinidae), in a Mediterranean stream

1. In the most metal-polluted reaches of Osor stream, Zn concentrations in water largely exceed the chronic toxicity threshold (120 µg Zn L^{-1}) for freshwater biota, so that Zn was the primary pollutant driving the differences in toxicity observed among stream reaches.
2. Periphyton significantly showed higher metal concentrations (mainly Zn, Mn and Fe) than macroinvertebrates, and metals did not biomagnify. Regarding the macroinvertebrate functional feeding groups, densities of grazers significantly decreased in the most polluted reaches, indicating that they were highly sensitive to water pollution.
3. Fish exclusion resulted in trophic cascade responses in the upper (Reference) reach, resulting in lower periphyton chlorophyll-*a* concentration due to higher grazing pressure. Conversely, in the metal-polluted reaches, fish exclusion revealed indirect positive effects of metal pollution on periphyton biomass by lowering grazing pressure.
4. Irrespective of metal pollution levels along the stream, the density of dipterans significantly decreased during the fish treatment, indicating strong predation effects on this taxon. To a lesser extent, variation in the density of EPT taxa and the total macroinvertebrate density approached statistical significance ($P < 0.1$). Interestingly, both periphyton clorophyll-*a* concentration and total biomass significantly decreased in fish treatments as a result of the bioturbation caused by the benthic fish behavior.
5. Because the presence of fish modified the indirect effects of metals on ecosystem structure, i.e. an increased periphyton biomass that would otherwise occur in their absence, it can be concluded that changes in fish density (*Barbus meridionalis*) can modify the effects of chemical pollution in streams.

GENERAL CONCLUSIONS

1. Receptor species matters: body size and morphological characteristics of fishes can act as major drivers of water velocity tolerance in fluvial ecosystems. Further, not all the macroinvertebrate communities and functional feeding groups responded equally to anthropogenic stressors. Macroinvertebrate grazers were the most responsive group to changes in discharge and water quality.
2. Reciprocal interactions: hydrologic disturbances can mitigate top-down (predator controlled) effects. By contrast, the absence of the top predator (*B. meridionalis*) triggered trophic cascades in benign and human-impacted streams, manifesting as changes in periphyton chlorophyll-*a* concentration and phosphate uptake.
3. This thesis has shed light on the complex interactions between environmental stressors and biological responses. This fact emphasizes the need for taking into account ecosystem-based and physiological approaches for understanding and predicting the future of fluvial ecosystems.

7. REFERENCES

A

Acuña V., Hunter M. and Ruhí A. (2017). Managing temporary streams and rivers as unique rather than second-class ecosystems. *Biological Conservation, 211*, 12–19.

Acuña V., Muñoz I., Giorgi A., Omella M., Sabater F. and Sabater S. (2005). Drought and postdrought recovery cycles in an intermittent Mediterranean stream: structural and functional aspects. *Journal of the North American Benthological Society, 24*, 919–933.

Adams S. R., Hoover J. J. and Killgore K. J. (1999). Swimming endurance of juvenile pallid sturgeon, *Scaphirhynchus albus*. *Copeia, 3*, 802–807.

Addelman S. (1969). The generalized randomized block design. *The American Statistician, 23*, 35–36.

Allan J. D. and Flecker A. S. (1993). Biodiversity conservation in running waters. *Bioscience, 43*, 32–43.

Alcaraz C., Bisazza A. and García-Berthou E. (2008a). Salinity mediates the competitive interactions between invasive mosquitofish and an endangered fish. *Oecologia, 155*, 205–213.

Alcaraz C., Pou-Rovira Q. and García-Berthou E. (2008b). Use of a flooded salt marsh habitat by an endangered cyprinodontid fish (*Aphanius iberus*). *Hydrobiologia, 600*, 177–185.

Alcaraz C. and García-Berthou E. (2007a). Life history variation of invasive mosquitofish (*Gambusia holbrooki*) along a salinity gradient. *Biological Conservation, 139*, 83–92.

Alcaraz C. and García-Berthou E. (2007b). Food of an endangered cyprinodont (*Aphanius iberus*): Ontogenetic diet shift and prey electivity. *Environmental Biology of Fishes, 78*, 193–207.

Alemadi S. D. and Jenkins D. G. (2008). Behavioral constraints for the spread of the eastern mosquitofish, *Gambusia holbrooki* (Poeciliidae). *Biological Invasions, 10,* 59–66.

Alexander A. C., Luis A. T., Culp J. M., Baird D. J. and Cessna A. J. (2013). Can nutrients mask community responses to insecticide mixtures? *Ecotoxicology, 22*, 1085–1100.

Alexandre C. M., Branca R., Quintella B. R. and Almeida P. R. (2016). Critical swimming speed of the southern straight-mouth nase *Pseudochondrostoma willkommii* (Steindachner, 1866), a potamodromous cyprinid from southern Europe. *Limnetica, 35,* 365–372.

Alexandre C. M., Quintella B. R., Ferreira A. F., Romão F. A. and Almeida P. R. (2014). Swimming performance and ecomorphology of the Iberian barbel *Luciobarbus bocagei* (Steindachner, 1864) on permanent and temporary rivers. *Ecology of Freshwater Fish, 23*, 244–258.

Allan J. D. (2004). Landscapes and riverscapes: The influence of land use on stream ecosystems. *Annual Review of Ecology, Evolution, and Systematics, 35,* 257–284.

Allan J. D. and Castillo M. (2007). Stream Ecology. Dordrecht, the Netherlands: Springer.

Allan J. D. and Flecker A. S. (1993). Biodiversity Conservation in Running Waters. *Bioscience, 43*, 32–43.

Allan J. D., Erickson D.L. and Fay J. (1997). The influence of catchment land use on stream integrity across multiple spatial scales. *Freshwater Biology, 37*, 149–161.

Anderson M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology, 26*, 32–46.

Anderson N. H. and Sedelll J. R. (1979). Detritus Processing by Macroinvertebrates in Stream Ecosystems. *Annual Review of Entomology, 24*, 351–377.

APHA. (1995). Standard Methods for the Examination of Water and Wastewater, Standard Methods (19th Edition). New York, USA: American Public Health Association.

Arenas-Sánchez A., Rico A. and Vighi M. (2016). Effects of water scarcity and chemical pollution in aquatic ecosystems: State of the art. *Science of the Total Environment, 572*, 390–403.

Argudo M. (2020). Microbial communities responses in fluvial biofilms under metal stressed scenarios. PhD Thesis. University of Girona, Spain.

Argudo M., Gich F., Bonet B., Espinosa C., Gutiérrez M. and Guasch H. (2020). Responses of resident (DNA) and active (RNA) microbial communities in fluvial biofilms under different polluted scenarios. *Chemosphere, 242*, 125108.

Arscott D. B., Larned S., Scarsbrook M. R. and Lambert P. (2010). Aquatic invertebrate community structure along an intermittence gradient: Selwyn River, New Zealand. *Journal of the North American Benthological Society*, *29*, 530–545.

Atli G., Guasch H., Rubio-Gracia F., Zamora L. and Vila-Gispert A. (2020). Antioxidant system status in threatened native fish *Barbus meridionalis* from the Osor River (Iberian Peninsula): I. Characterization and II. In vitro Zn assays. *Environmental Toxicology and Pharmacology*, 79, 103428.

Auer S. K., Salin K., Rudolf A. M., Anderson G. J. and Metcalfe N. B. (2015). The optimal combination of standard metabolic rate and aerobic scope for somatic growth depends on food availability. *Functional Ecology*, *29*, 479–486.

B

Barbee N. C. (2005). Grazing insects reduce algal biomass in a neotropical stream. *Hydrobiologia, 532*, 153–165.

Barbour M. T., Gerritsen J., Snyder B. D. and Stribling J. B. (1998). Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic

Macroinvertebrates and Fish (2nd edition). Washington D. C.: U. S. Environtal Protection Agency: Office of Water.

Bates D., Mächler M., Bolker B. M. and Walker S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software, 67*, 1–48.

Beamish F. W. H. (1978). Swimming capacity. In: Hoar W. S. and Randall D. J. (Eds.), *Fish physiology, locomotion*. Vol. VII. London, UK: Academic Press.

Beck W. S., Markman D. W., Oleksy I. A., Lafferty M. H. and Poff N. L. (2019). Seasonal shifts in the importance of bottom–up and top–down factors on stream periphyton community structure. *Oikos, 128*, 680–691.

Bell W. H. and Terhune L. D. B. (1970). Water tunnel design for fisheries research. *Fisheries Research Board of Canada Technical Report, 195*, 1–69.

Benejam L., Alcaraz C., Benito J., Caiola N., Casals F., et al. (2012). Fish catchability and comparison of four electrofishing crews in Mediterranean streams. *Fisheries Research, 123–124*, 9–15.

Benejam L., Alcaraz C., Sasal P., Simon-Levert G. and García-Berthou E. (2009). Life history and parasites of the invasive mosquitofish (*Gambusia holbrooki*) along a latitudinal gradient. *Biological Invasions, 11*, 2265–2277.

Bennett E. M., Carpenter S. R. and Caraco N. F. (2001). Human impact on erodable phosphorus and eutrophication: a global perspective. *Bioscience, 51*, 227–234.

Bennett A. F. and Ruben J. A. (1979). Endothermy and activity in invertebrates. *Science, 206*, 649–654.

Bernardo J. M., Ilhéu M., Matono P. and Costa A. M. (2003). Interannual variation of fish assemblage structure in a Mediterranean river: Implications of streamflow on the dominance of native or exotic species. *River Research and Applications, 19*, 521–532.

Biggs B. J. F., Francoeur S. N., Huryn A. D., Young R., Arbuckle C. J. and Townsend C. R. (2000). Trophic cascades in streams: effects of nutrient enrichment on autotrophic and consumer benthic communities under two different fish predation regimes. *Canadian Journal of Fisheries and Aquatic Sciences, 57*, 1380–1394.

Binning S. A. and Roche D. G. (2015). Water flow and fin shape polymorphism in coral reef fishes. *Ecology, 96*, 828–839.

Binning S. A., Ros A. F. H., Nusbaumer D. and Roche D. G. (2015). Physiological plasticity to water flow habitat in the damselfish, *Acanthochromis polyacanthus*: linking phenotype to performance. *PLoS ONE, 10*, e0121983.

Birk S. (2019). Detecting and Quantifying the Impact of Multiple Stress on River Ecosystems. In: Sabater, S., Elosegi A. and Ludwig R (Eds.), *Multiple Stressors in River Ecosystems*. 1st edition. Elsevier.

Biro P. A. and Stamps J. A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends in Ecology and Evolution, 25*, 653–659.

Blake R. W. (2004). Review paper: Fish functional design and swimming performance. *Journal of Fish Biology, 65*, 1193–1222.

Blanchet S., Loot G. and Dodson J. J. (2008). Competition, predation and flow rate as mediators of direct and indirect effects in a stream food chain. *Oecologia, 157*, 93–104.

Blueweiss L., Fox H., Kudzma V., Nakashima D., Peters R. and Sams S. (1978). Relationships between body size and some life history parameters. *Oecologia, 37*, 257–272.

Brett J. R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *Journal of the Fisheries Research Board of Canada, 21*, 1183–1226.

Boavida I., Santos J. M., Pinheiro A. N. and Ferreira M. T. (2011). Fish habitat availability simulations using different morphological variables. *Limnetica, 30*, 393–404.

Bochdansky A. B., Grønkjær P., Herra T. P. and Leggett W.C. (2005). Experimental evidence for selection against fish larvae with high metabolic rates in a food limited environment. *Marine Biology*, *147*, 1413–1417.

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

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

Bonet B., Corcoll N., Tlili A., Morin S. and Guasch H. (2014). Antioxidant enzyme activities in biofilms as biomarker of Zn pollution in a natural system: An active bio-monitoring study. *Ecotoxicology and Environmental Safety, 103*, 82–90.

Bonet B., Corcoll N., Acuña V., Sigg L., Behra R. and Guasch H. (2013). Seasonal changes in antioxidant enzyme activities of freshwater biofilms in a metal polluted Mediterranean stream. *Science of the Total Environment*, *444*, 1–10.

Bonnineau C., Artigas J., Chaumet B., Dabrin A., Faburé J., et al. (2020). Role of Biofilms in Contaminant Bioaccumulation and Trophic Transfer in Aquatic Ecosystems: Current State of Knowledge and Future Challenges. In: de Voogt P. (Ed.), *Reviews of Environmental Contamination and Toxicology (Continuation of Residue Reviews)*. Vol. 253. New York: Springer.

Borcard D., Legendre P. and Drapeau P. (1992). Partialling out the spatial component of ecological variation. *Ecology, 73*, 1045–1055.

Borer E. T., Seabloom E. W., Shurin J. B., Anderson K. E., Blanchette C. A., et al. (2005). What determines the strength of a trophic cascade? *Ecology, 86*, 528–537.

Bothwell M. (1988). Growth rate responses of lotic periphytic diatoms to experimental phosphorus enrichment: the influence of temperature and light. *Canadian Journal of Fisheries and Aquatic Science, 45*, 261–270.

Boyer E. W., Goodale C. L., Jaworski N. A. and Howarth R. W. (2002). Anthropogenic nitrogen sources and relationships to riverine nitrogen export in the northeastern U.S.A. *Biogeochemistry, 57*, 137–169.

Box G. E. and Cox D. R. (1964). An analysis of transformations revisited, rebutted. *Journal of the Royal Statistical Society: Series B, 26*, 211–252.

Brack W., Aissa S. A., Backhaus T., Dulio V., Escher B. I., et al. (2019). Effect-based methods are key. The European Collaborative Project SOLUTIONS recommends integrating effect-based methods for diagnosis and monitoring of water quality. *Environmental Sciences Europe, 31*, 10.

Bracken M. E. S. (2004). Invertebrate-mediated nutrient loading increases growth of an intertidal macroalga. *Journal of Phycology, 40*, 1032–1041.

Brashares J. S., Prugh L. R., Stoner C. J. and Epps C. W. (2010). Ecological and conservation implications of mesopredator release. In: Terborgh J. and Estes J. A. (Eds.), *Trophic cascades: predators, prey, and the changing dynamics of nature*. Washington: Island Press.

Brönmark C., Dahl J. and Greenberg L. A. (1997). Complex trophic interactions in freshwater benthic food chains. In: Streit B., Städler T. and Lively C. M. (Eds.), *Evolutionary Ecology of Freshwater Animals*. EXS, Vol. 82. Basel, Switzerland: Birkhäuser.

Brooks P. R. and Crowe T. P. (2018). Density and biotic interactions modify the combined effects of global and local stressors. *Oikos, 127*, 1746–1758.

Bruder A., Frainer A., Rota T. and Primicerio R. (2019). The importance of ecological networks in multiple-stressor research and management. *Frontiers in Environtal Science, 7,* 1–7.

Burton T., Killen, S. S., Armstrong J. D. and Metcalfe N. B. (2011). What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings of the Royal Society B: Biological Sciences, 278,* 3465–3473.

C

Cadmus P., Guasch H., Herdrich A. T., Bonet B., Urrea G. and Clements W. H. (2018). Structural and functional responses of periphyton and macroinvertebrate communities to ferric Fe, Cu, and Zn in stream mesocosms. *Environtal Toxicology and Chemistry, 37*, 1320–1329.

Cairns J., and Pratt J. R. (1993). A History of Biological Monitoring Using Benthic Macroinvertebrates. In: Rosenberg D. M. and Resh V. H. (Eds.), *Freshwater Biomonitoring and Benthic Macroinvertebrates*. New York: Chapman/Hall.

Calapez A. R., Elias C. L., Alves A., Almeida S. F. P., Brito A. G. and Feio M. J. (2020). Shifts in biofilms' composition induced by flow stagnation, sewage contamination and grazing. *Ecological Indicators, 111,* 106006.

Cano-Barbacil C., Radinger J., Argudo M., Rubio-Gracia F., Vila-Gispert A. and García-Berthou E. (2020a). Key factors explaining critical swimming speed in freshwater fish: a review and statistical analysis for Iberian species. *Scientific Reports, 10,* 18947.

Cano-Barbacil C., Radinger J. and García-Berthou E. (2020b). Reliability analysis of fish traits reveals discrepancies among databases. *Freshwater Biology, 65,* 863–877.

Capps K. A. and Flecker A. S. (2013). Invasive aquarium fish transform ecosystem nutrient dynamics. *Proceedings of the Royal Society B, 280,* 20131520.

Carmona-Catot G., Benito J. and García-Berthou E. (2011). Comparing latitudinal and upstream-downstream gradients: Life history traits of invasive mosquitofish. *Diversity and Distributions, 17,* 214–224.

Carmona-Catot G., Magellan K. and García-Berthou E. (2013). Temperature-specific competition between invasive mosquitofish and an endangered cyprinodontid fish. *PLoS ONE, 8,* e54734.

Carpenter S. R., Kitchell J. F. and Hodgson J. R. (1985). Cascading Trophic Interactions and Lake Productivity. *Bioscience, 35,* 634–639.

Cattaneo A., Kerimian T., Roberge M. and Marty J. (1997). Periphyton distribution and abundance on substrata of different size along a gradient of stream trophy. *Hydrobiologia, 354,* 101–110.

Chabot D., Steffensen J. F. and Farrell A. P. (2016). The determination of standard metabolic rate in fishes. *Journal of Fish Biology, 88,* 81–121.

Cheng B. S. and Grosholz E. D. (2016). Environmental stress mediates trophic cascade strength and resistance to invasion. *Ecosphere, 7,* e01247.

Clark T. D., Sandblom E. and Jutfelt F. (2013). Aerobic scope measurements of fishes in an era of climate change: Respirometry, relevance and recommendations. *Journal of Experimental Biology, 216,* 2771–2782.

Clavero M. and Hermoso V. (2011). Reservoirs promote the taxonomic homogenization of fish communities within river basins. *Biodiversity and Conservation, 20,* 41–57.

Clements W. H. (1999). Metal Tolerance and Predator-Prey Interactions in Benthic Macroinvertebrate Stream Communities. *Ecological Applications, 9,* 1073–1084.

Clements W. H. and Rohr J. R. (2009). Community responses to contaminants: using basic ecological principles to predict ecotoxicological effects. *Environmental Toxicology and Chemistry, 28*, 1789–1800.

Clements W. H. and Rees D. E. (1997). Effects of Heavy Metals on Prey Abundance, Feeding Habits, and Metal Uptake of Brown Trout in the Arkansas River, Colorado. *Transactions of the American Fisheries Society, 126*, 774–785.

Clements W. H., Kashian D. R., Kiffney P. M. and Zuellig R. E. (2016). Perspectives on the context-dependency of stream community responses to contaminants. *Freshwater Biology, 61*, 2162–2170.

Clements W. H., Carlisle D. M., Courtney L. A. and Harrahy E. A. (2002). Integrating observational and experimental approaches to demonstrate causation in stream biomonitoring studies. *Environmental Toxicology and Chemistry, 21*, 1138–1146.

Clements W. H., Carlisle D. M., Lazorchak J. M. and Johnson P. C. (2000). Heavy metals structure benthic communities in Colorado mountain streams. *Ecological Applications, 10*, 626–638.

Connon R. E., Geist J. and Werner I. (2012). Effect-based tools for monitoring and predicting the ecotoxicological effects of chemicals in the aquatic environment. *Sensors (Basel), 12*, 12741–12771.

Conradsen C. and McGuigan K. (2015). Sexually dimorphic morphology and swimming performance relationships in wild-type zebrafish *Danio rerio*. *Journal of Fish Biology, 87*, 1219–1233.

Corcoll N., Bonet B., Morin S., Tlili A., Leira M. and Guasch H. (2012). The effect of metals on photosynthesis processes and diatom metrics of biofilm from a metal-contaminated river: a translocation experiment. *Ecological Indicators*, *18*, 620–631.

Costa M. J., Lennox R. J., Katopodis C. and Cooke S. J. (2017). Is there evidence for flow variability as an organism-level stressor in fluvial fish? *Journal of Ecohydraulics, 2,* 68–83.

Courtney L. A. and Clements W. H. (2002). Assessing the influence of water and substratum quality on benthic macroinvertebrate communities in a metal-polluted stream: An experimental approach. *Freshwater Biology, 47*, 1766–1778.

Crain C. M. and Bertness M. D. (2006). Ecosystem engineering across environmental gradients: Implications for conservation and management. *Bioscience*, *56*, 211–218.

D

Dahl J. (1998). Effects of a benthivorous and a drift-feeding fish on a benthic stream assemblage. *Oecologia, 116*, 426–432.

Davis J. M., Rosemond A. D., Eggert S. L., Cross W. F. and Wallace J. B. (2010). Long-term nutrient enrichment decouples predator and prey production. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 121–126

De Castro-Català N., Dolédec S., Kalogianni E., Skoulikidis N. T., Paunovic M., et al. (2020). Unravelling the effects of multiple stressors on diatom and macroinvertebrate communities in European river basins using structural and functional approaches. *Science of the Total Environment, 742*, 140543.

De Schamphelaere K. A. C. and Janssen C. R. (2004). Bioavailability and chronic toxicity of zinc to juvenile rainbow trout (*Oncorhynchus mykiss*): Comparison with other fish species and development of a biotic ligand model. *Environtal Science & Technology, 38*, 6201–6209.

DeLong J. P., Gilbert B., Shurin J. B., Savage V. M., Barton B. T., et al. (2015). The body size dependence of trophic cascades. *The American Naturalist, 185,* 354–366.

Del Signore A., Lenders H. J. R., Hendriks A. J., Vonk J. A., Mulder C. and Leuven R. S. E. W. (2016). Size-Mediated Effects of Water-Flow Velocity on Riverine Fish Species. *River Research and Applications, 32*, 390–398.

Díez-del-Molino D., García-Berthou E., Araguas R. M., Alcaraz C., Vidal O., Sanz N. and García-Marín J. L. (2018). Effects of water pollution and river fragmentation on population genetic structure of invasive mosquitofish. *Science of the Total Environment, 637–638*, 1372–1382.

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

Dodds W. K. (2003). The role of periphyton in phosphorous retention in shallow freshwater aquatic systems. *Journal of Phycology, 39*, 840–849.

Dodds W. K. (2002). Freshwater Ecology. San Diego: Academic Press.

Domenici P., Turesson H., Brodersen J. and Brönmark C. (2008). Predator-induced morphology enhances escape locomotion in Crucian carp. *Proceedings of the Royal Society B: Biological* Sciences, 275, 195–201.

Dudgeon D., Arthington A. H., Gessner M. O., Kawabata Z. I., Knowler D. J., et al. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews of the Cambridge Philosophical Society, 81*, 163–182.

E

Elosegi A. and Sabater S. (2009). Conceptos y técnicas en ecología fluvial. Fundacion BBVA.

Elser J. J., Bracken M. E. S., Cleland E. E., Gruner D. S., Harpole W. S., et al. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology letters, 10,* 1135–1142.

Environmental Protection Agency of United States, EPA. (2006). National Recommended Water Quality Criteria Table: Poster and Brochure. Available from: http://water.epa.gov/type/rsl/; 2006.

Erman D. C., Andrews E. D. and Yoder-Williams M. (1988). Effects of winter floods on fishes in the Sierra Nevada. *Canadian Journal of Fisheries and Aquatic Sciences, 45,* 2195–2200.

Evans-White M. A. and Lamberti G. A. (2005). Grazer species effects on epilithon nutrient composition. *Freshwater Biology, 50,* 1853–1863.

F

Farag A. M., Woodward D. F., Goldstein J. N., Brumbaugh W. and Meyer J. S. (1998). Concentrations of metals associated with mining waste in sediments, biofilm, benthic macroinvertebrates, and fish from the Coeur d'Alene River Basin, Idaho. *Archives of Environmental Contamination and Toxicology, 34,* 119–127.

Farrell A. P. (2008). Comparisons of swimming performance in rainbow trout using constant acceleration and critical swimming speed tests. *Journal of Fish Biology, 72,* 693–710.

Feld C. K., Martins da Silva P., Paulo Sousa J., De Bello F., Bugter R., et al. (2009). Indicators of biodiversity and ecosystem services: a synthesis across ecosystems and spatial scales. *Oikos, 118*, 1862–1871.

Feminella J. W. (1996). Comparison of benthic macroinvertebrate assemblages in small streams along a gradient of flow permanence. *Journal of the North American Benthological Society, 15*, 651–669.

Feminella J. W. and Hawkins C. P. (1995). Interactions between stream herbivores and periphyton: a quantitative analysis of past experiments. *Journal of the North American Benthological Society, 14*, 465–509.

Filipe A. F., Lawrence J. E. and Bonada N. (2013). Vulnerability of stream biota to climate change in mediterranean climate regions: A synthesis of ecological responses and conservation challenges. *Hydrobiologia, 719*, 331–351.

Fischer J. R. and Paukert C. P. (2008). Habitat relationships with fish assemblages in minimally disturbed Great Plains regions. *Ecology of Freshwater Fish, 17*, 597–609.

Fisher R. and Hogan J. D. (2007). Morphological predictors of swimming speed: A case study of pre-settlement juvenile coral reef fishes. *Journal of Experimental Biology, 210*, 2436–2443.

Fisher R., Leis J. M., Clark D. L. and Wilson C. S. (2005). Critical swimming speeds of late-stage coral reef fish larvae: variation within species, among species and between locations. *Marine Biology, 147*, 1201–1212.

Fisher R., Bellwood D. R. and Job S. D. (2000). Development of swimming abilities in reef fish larvae. *Marine Ecology Progress Series, 202*, 163–173.

Flecker A. S. (1996). Ecosystem engineering by a dominant detritivore in a diverse tropical stream. *Ecology, 77*, 1845–1854.

Flecker A. S. and Taylor B. W. (2004). Tropical fishes as biological bulldozers: density effects on resource heterogeneity and species diversity. *Ecology, 85*, 2267–2278.

Flecker A. S. and Townsend C. R. (1994). Community-Wide Consequences of Trout Introduction in New Zealand Streams. *Ecological Applications, 4*, 798–807.

Fleeger J. W., Tita G., Carman K. R., Millward R. N., Moser E. B., et al. (2006). Does bioturbation by a benthic fish modify the effects of sediment contamination on saltmarsh benthic microalgae and meiofauna? *Journal of Experimental Marine Biology and Ecology, 330*, 180–194.

Fleeger J. W., Carman K. R. and Nisbet R. M. (2003). Indirect effects of contaminants in aquatic ecosystems. *Science of the Total Environment, 317*, 207–233.

Frimpong E. A. and Angermeier P. L. (2010). Trait-based approaches in the analysis of stream fish communities. *American Fisheries Society Symposium, 73*, 109–136.

Fritz K. M. and Dodds W. K. (2004). Resistance and resilience of macroinvertebrate assemblages to drying and flood in a tallgrass prairie stream system. *Hydrobiologia, 527*, 99–112.

Fritz K. M. and Dodds W. K. (2002). Macroinvertebrate assemblage structure across a tallgrass prairie stream landscape. *Archiv für Hydrobiologie, 154*, 79–102.

Fry F. E. J. (1971). The effect of environmental factors on the physiology of fish. *Fish Physiology, 6*, 1–98.

Fry F. E. J. and Hart J. S. (1948). The relation of temperature to oxygen consumption in the goldfish. *The Biological Bulletin, 94*, 66–77.

Fulton C. J. (2007). Swimming speed performance in coral reef fishes: field validations reveal distinct functional groups. *Coral Reefs, 26*, 217–228.

G

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

García-Berthou E. and Moreno-Amich R. (1992). Age and growth of an Iberian cyprinodont. *Journal of Fish Biology, 40*, 929–937.

Gartzia De Bikuña B., López E., Leonardo J. M., Arrate J., Martínez A., et al. (2015). Reduction of sampling effort assessing macroinvertebrate assemblages for biomonitoring of rivers. *Knowledge & Management of Aquatic Ecosystems, 416*, 08.

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

Geist J. (2011). Integrative freshwater ecology and biodiversity conservation. *Ecological Indicators, 11*, 1507–1516.

Ghedini G., Russell B. D. and Connell S.D. (2015). Trophic compensation reinforces resistance: herbivory absorbs the increasing effects of multiple disturbances. *Ecology Letters, 18*, 182–187.

Gido K. B., Propst D. L., Olden J. D. and Bestgen K. R. (2013). Multidecadal responses of native and introduced fishes to natural and altered flow regimes in the American Southwest. *Canadian Journal of Fisheries and Aquatic Sciences, 70*, 554–564.

Gido K. B., Bertrand K. N., Murdock J. N., Dodds W. K. and Whiles M. R. (2010). Disturbance-mediated effects of fishes on stream ecosystem processes: concepts and results from highly variable prairie streams. *American Fisheries Society Symposium, 73*, 593–617.

Gonzalez E. G., Cunha C., Ghanavi H. R., Oliva-Paterna F. J., Torralva M. and Doadrio I. (2018). Phylogeography and population genetic analyses in the iberian toothcarp (*Aphanius iberus* Valenciennes, 1846) at different time scales. *Journal of Heredity, 109,* 253–263.

Goodyear K. L. and McNeill S. (1999). Bioaccumulation of heavy metals by aquatic macro-invertebrates of different feeding guilds: A review. *Science of the Total Environment, 229,* 1–19.

Gruner D. S., Smith J. E., Seabloom E. W., Sandin S. A., Ngai J. T., et al. (2008). A cross-system synthesis of consumer and nutrient resource control on producer biomass. *Ecology Letters, 11*, 740–755.

Guasch H. and Sabater S. (1994). Primary production of epilithic communities in undisturbed Mediterranean streams. *Verhandlungen des Internationalen Verein Limnologie, 25,* 1761–1764.

Guasch H., Martí E. and Sabater S. (1995). Nutrient enrichment effects on biofilm metabolism in a Mediterranean stream. *Freshwater Biology, 33,* 373–383.

Guasch H., Ricart M., López-Doval J., Bonnineau C., Proia L., et al. (2016). Influence of grazing on triclosan toxicity to streamperiphyton. *Freshwater Biology, 61,* 2002–2012.

Guasch H., Artigas J., Bonet B., Bonnineau C., Canals O., et al. (2016). The Use of Biofilms to Assess the Effects of Chemicals on Freshwater Ecosystems. In: Romaní A. M., Guasch H. and Balaguer M. D. (Eds.), *Aquatic Biofilms: Ecology, Water Quality and Wastewater Treatment*. Norfolk, UK: Caister Academic Press.

Guasch H., Bonet B., Bonnineau C., Corcoll N., López-Doval J. C., et al. (2012). How to Link Field Observations with Causality? Field and Experimental Approaches Linking Chemical Pollution with Ecological Alterations. In: Guasch H., Ginebreda A. and

Geiszinger A. (Eds.), *Emerging and Priority Pollutants in Rivers. The Handbook of Environmental Chemistry*. Vol 19. Berlin, Heidelberg: Springer.

Guasch H., Atli G., Bonet B., Corcoll N., Leira M. and Serra A. (2010). Discharge and the response of biofilms to metal exposure in Mediterranean rivers. *Hydrobiologia, 657*, 143–157.

Guasch H., Serra A., Corcoll N., Bonet B. and Leira M. (2009). Metal Ecotoxicology in Fluvial Biofilms: Potential Influence of Water Scarcity. In: Sabater S. and Barceló D. (Eds.), *Water Scarcity in the Mediterranean. The Handbook of Environmental Chemistry*. Vol 8. Berlin, Heidelberg: Springer.

Guderley H. and Pörtner H. O. (2010). Metabolic power budgeting and adaptive strategies in zoology: examples from scallops and fish. *Canadian Journal of Zoology, 88*, 753–763.

H

Haglund A. L. and Hillebrand H. (2005). The effect of grazing and nutrient supply on periphyton associated bacteria. *FEMS Microbiology Ecology, 52*, 31–41.

Halsey L. G., Killen S. S., Clark T. D. and Norin T. (2018). Exploring key issues of aerobic scope interpretation in ectotherms: Absolute versus factorial. *Reviews in Fish Biology and Fisheries, 28*, 405–415.

Hamilton S. K., Bruesewitz D. A., Horst G. P., Weed D. B. and Sarnelle O. (2009). Biogenic calcite–phosphorus precipitation as a negative feedback to lake eutrophication. *Canadian Journal of Fisheries and Aquatic Sciences, 66*, 343–350.

Hammer C. (1995). Fatigue and exercise tests with fish. *Comparative Biochemistry and Physiology A, 112*, 1–20.

Haro A., Castro-Santos T., Noreika J. and Odeh M. (2004). Swimming performance of upstream migrant fishes in open-channel flow: a new approach to predicting passage through velocity barriers. *Canadian Journal of Fisheries and Aquatic Sciences, 61,* 1590–1601.

Harvey B. C. (1987). Susceptibility of young-of-the-year fishes to downstream displacement by flooding. *Transactions of the American Fisheries Society, 116,* 851–855.

Hepher B. (1988). Nutrition of Pond Fishes. Cambridge, UK: Cambridge University Press.

Hill B. H., Willingham W. T., Parrish L. P. and McFarland B. H. (2000). Periphyton community responses to elevated metal concentrations in a Rocky Mountain stream. *Hydrobiologia, 428,* 161–169.

Hillebrand H. (2005). Light regime and consumer control of autotrophic biomass. *Journal of Ecology, 93,* 758–769.

Hillebrand H and Kahlert M. (2001). Effect of grazing and nutrient supply on periphyton biomass and nutrient stoichiometry in habitats of different productivity. *Limnology and Oceanography, 46,* 1881–1898.

Hillebrand H. and Sommer U. (1999). The nutrient stoichiometry of benthic microalgal growth: Redfield proportions are optimal. *Limnology and Oceanography, 44***,** 440–446.

Hillebrand H., Kahlert M., Haglund A. L., Berninger U. G., Nagel S. and Wickham S. (2002). Control of microbenthic communities by grazing and nutrient supply. *Ecology, 83,* 2205–2219.

Hockley F. A., Wilson C. A. M. E., Brew A. and Cable J. (2014). Fish responses to flow velocity and turbulence in relation to size, sex and parasite load. *Journal of the Royal Society Interface, 11,* 20130814.

Hogan J. D., Fisher R. and Nolan C. (2007). Critical swimming speed of settlement-stage coral reef fishes from the Caribbean: a methodological and geographical comparison. *Bulletin of Marine Science, 80*, 219–231.

Holomuzki J. R., Feminella J. W. and Power M. E. (2010). Biotic interactions in freshwater benthic habitats. *Journal of the North American Benthological Society, 29*, 220–244.

Humphries P., Brown P., Douglas J., Pickworth A., Strongman R., et al. (2008). Flow-related patterns in abundance and composition of the fish fauna of a degraded Australian lowland river. *Freshwater Biology, 53*, 789–813.

Hunter M. D. and Price P. W. (1992). Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology, 73*, 723–732.

Hurlbert S. H. (1971). The nonconcept of species diversity: a critique and alternative parameters. *Ecology, 52*, 577–586.

I

Intergovernmental Panel on Climate Change, IPCC (2007). Climate Change 2007 – The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the IPCC. Intergovernmental Panel on Climate Change.

Iwasaki Y., Kagaya T., Miyamoto K. I. and Matsuda H. (2009). Effects of heavy metals on riverine benthic macroinvertebrate assemblages with reference to potential food availability for drift-feeding fishes. *Environmental Toxicology and Chemistry, 28*, 354–363.

J

Jansen L. S., O’Dowd A. and Bouma-Gregson K. (2020). A comparison of benthic algal and macroinvertebrate communities in a dammed and undammed Mediterranean river (Eel River watershed, California, USA). *River Research and Applications, 36*, 1668–1681.

Jeffrey S. W. and Humphrey G. F. (1975). New spectrophotometric equations for determining chlorophylls *a*, *b*, *c*1 and *c*2 in higher plants, algae and natural phytoplankton. *Biochemie und Physiologie der Pflanzen, 167,* 191–194.

Jellyman P. G. and McIntosh A. R. (2020). Disturbance-mediated consumer assemblages determine fish community structure and moderate top-down influences through bottom-up constraints. *Journal of Animal Ecology, 89*, 1175–1189.

Jones C. G., Lawton J. H. and Shachak M. (1997). Positive and Negative Effects of Organisms as Physical Ecosystem Engineers. *Ecology, 78*, 1946–1957.

Jones E. A., Lucey K. S. and Ellerby D. J. (2007). Efficiency of labriform swimming in the bluegill sunfish *Lepomis macrochirus*. *Journal of Experimental Biology, 210*, 3422–3429.

Jones P. E., Svendsen J. C., Börger L., Champneys T., Consuegra S., et al. (2020). One size does not fit all: inter- and intraspecific variation in the swimming performance of contrasting freshwater fish. *Conservation Physiology, 8*, coaa126.

K

Kalogianni E., Vourka A., Karaouzas I., Vardakas L., Laschou S. and Skoulidikis N. Th. (2017). Combined effects of water stress and pollution on macroinvertebrate and fish assemblages in a Mediterranean intermittent river. *Science of the Total Environment, 603–604,* 639–650.

Karaouzas I., Smeti E., Vourka A., Vardakas L., Mentzafou A., et al. (2018). Assessing the ecological effects of water stress and pollution in a temporary river - Implications for water management. *Science of the Total Environment, 618,* 1591–1604.

Katopodis C. (1992). Introduction to Fishway Design. Winnipeg, Manitoba: Department of Fisheries and Oceans.

Katopodis C., Cai L. and Johnson D. (2019). Sturgeon survival: The role of swimming performance and fish passage research. *Fisheries Research, 212,* 162–171.

Keefer M., Moser M. L., Boggs C. T., Daigle W. R. and Peery C. A. (2009). Effects of Body Size and River Environment on the Upstream Migration of Adult Pacific Lampreys. *North American Journal of Fisheries Management, 29,* 1214–1224.

Keen J. E. and Farrell A. P. (1994). Maximum prolonged swimming speed and maximum cardiac performance of rainbow trout, *Oncorhynchus mykiss*, acclimated to two different water temperatures. *Comparative Biochemistry and Physiology A, 108,* 287–295.

Kendall J. L., Lucey K. S., Jones E. A., Wang J. and Ellerby D. J. (2007). Mechanical and energetic factors underlying gait transitions in bluegill sunfish (*Lepomis macrochirus*). *Journal of Experimental Biology, 210,* 4265–4271.

Kennelly S. J. (1991). Caging experiments to examine the effects of fishes on understorey species in a sublittoral kelp community. *Journal of Experimental Marine Biology and Ecology, 147,* 207–230.

Kiffney P. M. (1996). Main and interactive effects of invertebrate density, predation, and metals on a Rocky Mountain stream macroinvertebrate community. *Canadian Journal of Fisheries and Aquatic Sciences, 53,* 1595–1601.

Killen S. S., Norin T. and Halsey L. G. (2017). Do method and species lifestyle affect measures of maximum metabolic rate in fishes? *Journal of Fish Biology, 90*, 1037–1046.

Killen S. S., Croft D. P., Salin K. and Darden S. K. (2016). Male sexually coercive behaviour drives increased swimming efficiency in female guppies. *Functional Ecology, 30*, 576–583.

Killen S. S., Glazier D. S., Rezende E. L., Clark T. D., Atkinson D., et al. (2016). Ecological influences and morphological correlates of resting and maximal metabolic rates across teleost fish species. *The American Naturalist, 187,* 592–606.

Killen S. S. (2014). Growth trajectory influences temperature preference in fish through an effect on metabolic rate. *Journal of Animal Ecology, 83*, 1513–1522.

Killen S. S., Atkinson D. and Glazier D. S. (2010). The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. *Ecology Letters, 13,* 184–193.

Killen S. S., Costa I., Brown J. A. and Gamperl A. K. (2007). Little left in the tank: metabolic scaling in marine teleosts and its implications for aerobic scope. *Proceedings of the Royal Society B: Biological Sciences, 274*, 431–438.

Kim K. S., Funk D. H. and Buchwalter D. B. (2012). Dietary (periphyton) and aqueous Zn bioaccumulation dynamics in the mayfly *Centroptilum triangulifer*. *Ecotoxicology, 21,* 2288–2296.

Klemmer A. J. and Richardson J. S. (2013). Quantitative gradient of subsidies reveals a threshold in community-level trophic cascades. *Ecology, 94*, 1920–1926.

Kolok A. S. (1999). Interindividual variation in the prolonged locomotor performance of ectothermic vertebrates: a comparison of fish and herpetofaunal methodologies and a brief review of the recent fish literature. *Canadian Journal of Fisheries and Aquatic Science, 56,* 700–710.

Korpinen S., Jormalainen V. and Honkanen T. (2007). Bottom-up and cascading top-down control of macroalgae along a depth gradient. *Journal of Experimental Marine Biology and Ecology, 343*, 52–63.

Korsmeyer K. E., Steffensen J. F. and Herskin J. (2002). Energetics of median and paired fin swimming, body and caudal fin swimming, and gait transition in parrotfish (*Scarus schlegeli*) and triggerfish (*Rhinecanthus aculeatus*). *Journal of Experimental Biology, 205*, 1253–1263.

Kotalik C. J. and Clements W. H. (2019). Stream mesocosm experiments show significant differences in sensitivity of larval and emerging adults to metals. *Environtal Science & Technology, 53*, 8362–8370.

Kuznetsova A., Brockhoff P. B. and Christensen R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software, 82*, 1–26.

L

Lake P. S. (2003). Ecological effects of perturbation by drought in flowing waters. *Freshwater Biology, 48*, 1161–1172.

Lamberti G. A., Chaloner D. T. and Hershey A. E. (2010). Linkages among aquatic ecosystems. *Journal of the North American Benthological Society, 29*, 245–263.

Langerhans R. B. (2009). Trade-off between steady and unsteady swimming underlies predator-driven divergence in *Gambusia affinis*. *Journal of Evolutionary Biology, 22*, 1057–1075.

Langerhans R. and Reznick D. (2010). Ecology and evolution of swimming performance in fishes: Predicting evolution with biomechanics. In: Domenici P. and Kapoor B. G.

(Eds.), *Fish locomotion*: An ethoecological perspective. Enfield, UK: Science Publishers.

Larned S. T., Datry T., Arscott D. B. and Tockner K. (2010). Emerging concepts in temporary-river ecology. *Freshwater Biology, 55*, 717–738.

Larsen S., Muehlbauer J. D. and Marti E. (2016). Resource subsidies between stream and terrestrial ecosystems under global change. *Global change biology, 22*, 2489–2504.

Layer K., Hildrew A. G. and Woodward G. (2013). Grazing and detritivory in 20 stream food webs across a broad pH gradient. *Oecologia, 171*, 459–471.

Layer K., Riede J. O., Hildrew A. G. and Woodward G. (2010). Food Web Structure and Stability in 20 Streams Across a Wide pH Gradient. In: Woodward G. (Ed.), *Advances in Ecological Research*. Vol 42. London, UK: Academic Press.

Leavy T. R. and Bonner T. H. (2009). Relationships among swimming ability, current velocity association, and morphology for freshwater lotic fishes. *North American Journal of Fisheries Management, 29*, 72–83.

Ledger M. E. and Hildrew A. G. (2005). The ecology of acidification and recovery: Changes in herbivore-algal food web linkages across a stream pH gradient. *Environmental Pollution, 137*, 103–118.

Leroux S. J. and Loreau M. (2008). Subsidy hypothesis and strength of trophic cascades across ecosystems. *Ecology Letters, 11*, 1147–1156.

Li J., Lin X., Xu Z. and Sun J. (2017). Differences in swimming ability and its response to starvation among male and female *Gambusia affinis*. *Biology Open, 6*, 625–632.

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

Ludlam J. P. and Magoulick D. D. (2010). Environmental conditions and biotic interactions influence ecosystem structure and function in a drying stream. *Hydrobiologia, 644*, 127–137.

M

Magellan K. and García-Berthou E. (2016). Experimental evidence for the use of artificial refugia to mitigate the impacts of invasive *Gambusia holbrooki* on an endangered fish. *Biological Invasions, 18*, 873–882.

Magoulick D. D. and Kobza R. M. (2003). The role of refugia for fishes during drought: a review and synthesis. *Freshwater Biology, 48*, 1186–1198.

Maret T. R., Cain D. J., MacCoy D. E. and Short T. M. (2003). Response of benthic invertebrate assemblages to metal exposure and bioaccumulation associated with hard-rock mining in northwestern streams, USA. *Journal of the North American Benthological Society, 22*, 598–620.

Marqués M. J., Martínez-Conde E. and Rovira J. V. (2003). Effects of zinc and lead mining on the benthic macroinvertebrates of a fluvial ecosystem. *Water, Air, & Soil Pollution, 148*, 363–388.

Martí E., Aumatell J., Godé L., Poch M. and Sabater F. (2001). Nutrient retention efficiency in streams receiving inputs from wastewater treatment plants. *Journal of Environmental Quality, 33*, 285–293.

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

Mateus C. S., Quintella B. R. and Almeida P. R. (2008). The critical swimming speed of Iberian barbel *Barbus bocagei* in relation to size and sex. *Journal of Fish Biology, 73*, 1783–1789.

Mathers K. L., Stubbington R., Leeming D., Westwood C. and England J. (2019). Structural and functional responses of macroinvertebrate assemblages to long-term flow variability at perennial and nonperennial sites. *Ecohydrology, 12,* e2112.

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

McAfee D. and Bishop M. J. (2019). The mechanisms by which oysters facilitate invertebrates vary across environmental gradients. *Oecologia, 189*, 1095–1106.

McIntyre P. B., Flecker A. S., Vanni M. J., Hood J. M., Taylor B. W. and Thomas S. A. (2008). Fish distributions and nutrient cycling in streams: can fish create biogeochemical hotspots? *Ecology, 89,* 2335–2346.

Mebane C. A., Schmidt T. S. and Balistrieri L. S. (2017). Larval aquatic insect responses to cadmium and zinc in experimental streams. *Environmental Toxicology & Chemistry, 36*, 749–762.

Medley C. N. and Clements W. H. (1998). Responses of diatom communities to heavy metals in streams: The influence of longitudinal variation. *Ecological Applications, 8*, 631–644.

Meißner T., Sures B. and Feld C. K. (2019). Multiple stressors and the role of hydrology on benthic invertebrates in mountainous streams. *Science of the Total Environment, 663,* 841–851.

Menge B. A. and Sutherland J. P. (1987). Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. *The American Naturalist, 130*, 730–757.

Merbt S. N., Auguet J.-C., Casamayor E. O. and Martí E. (2011). Biofilm recovery in a wastewater treatment plant-influenced stream and spatial segregation of ammonia-oxidizing microbial populations. *Limnology and Oceanography, 56*, 1054–1064.

Merbt S. N., Bernal S., Proia L., Martí E. and Casamayor E. O. (2017). Photoinhibition on natural ammonia oxidizers biofilm populations and implications for nitrogen uptake in stream biofilms. *Limnology and Oceanography, 62*, 364–375.

Merciai R., Bailey L. L., Bestgen K. R., Fausch K., Zamora L., et al. (2018). Water diversion reduces abundance and survival of two Mediterranean cyprinids. *Ecology of Freshwater Fish, 27*, 1054–1064.

Merciai R., Molons-Sierra C., Sabater S. and García-Berthou E. (2017). Water abstraction affects abundance, size-structure and growth of two threatened cyprinid fishes. *PLoS One*, 12: e0175932.

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

Metcalfe N. B., Van Leuuwen T. E. and Killen S. S. (2016). Does individual variation in metabolic phenotype predict fish behaviour and performance? *Journal of Fish Biology, 88*, 298–321.

Minckley W. L. and Meffe G. K. (1987). Differential selection by flooding in streamfish communities of the arid American southwest. In: Matthews W. J. and Heins D. C. (Eds.), *Community and evolutionary ecology of North American stream fishes*. Norman: University of Oklahoma Press.

Minshall G. W. (1978). Autotrophy in Stream Ecosystems. *BioScience, 28*, 767–771.

Moore J. C., Berlow E. L., Coleman D. C., De Suiter P. C., Dong Q., et al. (2004). Detritus, trophic dynamics and biodiversity. *Ecology Letters, 7*, 584–600.

Moore J. W. (2006). Animal ecosystem engineers in streams. *Bioscience, 56*, 237–246.

Moore J. W., Schindler D. E. and Scheuerell M. D. (2004). Disturbance of freshwater habitats by anadromous salmon in Alaska. *Oecologia, 139*, 298–308.

Moran C. J., Ferry L. A. and Gibb A. C. (2016). Why does *Gila elegans* have a bony tail? A study of swimming morphology convergence. *Zoology, 119*, 175–181.

Morin S., Duong T. T., Dabrin A., Coynel A., Herlory O., et al. (2008). Long-term survey of heavy-metal pollution, biofilm contamination and diatom community structure in the Riou Mort watershed, South-West France. *Environmental Pollution, 151*, 532–542.

Morin S., Vivas-Nogues M., Duong T. T., Boudou A. and Delmas F. (2007). Dynamics of benthic diatom colonization in a cadmium/zinc-polluted river (Riou-Mort, France). *Fundamental and Applied Limnology, 168*, 179–187.

Moulton T. P., Souza M. L., Silveira R. M. L., Krsulović F. A. M., Silveira M. P., et al. (2010). Patterns of periphyton are determined by cascading trophic relationships in two neotropical streams. *Marine & Freshwater Research, 61*, 57–64.

Moyle P. B. and Cech J. J. (1988). Fish: An Introduction to Ichthyology. 2nd Edition. Englewood Cliffs: Prentice-Hall.

Mueller M., Pander J. and Geist J. (2011). The effects of weirs on structural stream habitat and biological communities. *Journal of Applied Ecology, 48*, 1450–1461.

Mulholland P. J., Helton A. M., Poole G. C., Hall R. O., Hamilton S. K., et al. (2008). Stream denitrification across biomes and its response to anthropogenic nitrate loading. *Nature, 452*, 202–205.

Mulholland P. J., Steinman A. D., Palumbo A. V. and Elwood J. W. (1991). Role of nutrient cycling and herbivory in regulating periphyton communities in laboratory streams. *Ecology, 72*, 966–982.

Murdock J. N., Dodds W. K., Gido K. B. and Whiles M. R. (2011). Dynamic influences of nutrients and grazing fish on periphyton during recovery from flood. *Journal of the North American Benthological Society, 30,* 331–345.

Murphy J. and Riley J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta, 27,* 31–36.

N

Naiman R. J., Latterell J. J., Pettit N. E. and Olden J. D. (2008). Flow variability and the biophysical vitality of river systems. *Comptes Rendus - Geoscience, 340*, 629–643.

Nakagawa H. (2014). Contribution of environmental and spatial factors to the structure of stream fish assemblages at different spatial scales. *Ecology of Freshwater Fish, 23,* 208–223.

Nakagawa S. and Schielzeth H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution, 4*, 133–142.

Nanami A. (2007). Juvenile swimming performance of three fish species on an exposed sandy beach in Japan. *Journal of Experimental Marine Biology and Ecology, 348,* 1–10.

Nelson J. A. (2016). Oxygen consumption rate v. rate of energy utilization of fishes: A comparison and brief history of the two measurements. *Journal of Fish Biology, 88,* 10–25.

Noonan M. J., Grant J. W. A. and Jackson C. D. (2012). A quantitative assessment of fish passage efficiency. *Fish and Fisheries, 13*, 450–464.

Norin T. and Clark T. D. (2016). Measurement and relevance of maximum metabolic rate in fishes. *Journal of Fish Biology, 88,* 122–151.

Norin T. and Malte H. (2012). Intraspecific variation in aerobic metabolic rate of fish: relations with organ size and enzyme activity in brown trout. *Physiological and Biochemical Zoology, 85*, 645–656.

O

O'Brien J. M. and Dodds W. K. (2008). Ammonium uptake and mineralization in prairie streams: chamber incubation and short-term nutrient addition experiments. *Freshwater Biology, 53*, 102–112.

Odum E. P. and Biever L. J. (1984). Resource quality, mutualism, and energy partitioning in food chains. *The American Naturalist, 124*, 360–376.

Ohlberger J., Staaks G. and Hölker F. (2007). Effects of temperature, swimming speed and body mass on standard and active metabolic rate in vendace *Coregonus albula*. *Journal of Comparative Physiology B, 177*, 905–916.

Ohlberger J., Staaks G. and Hölker F. (2006). Swimming efficiency and the influence of morphology on swimming costs in fishes. *Journal of Comparative Physiology B, 176*, 17–25.

Ohlberger J., Staaks G., Van Dijk P. L. M. and Hölker F. (2005). Modelling energetic costs of fish swimming. *Journal of Experimental Zoology Part A: Comparative Experimental Biology, 303*, 657–664.

Ojanguren A. F. and Braña F. (2003). Effects of size and morphology on swimming performance in juvenile brown trout *(Salmo trutta* L.). *Ecology of Freshwater Fish, 12*, 241–246.

Oksanen J., Blanchet F. G., Friendly M., Kindt R., Legendre P., et al. (2017). Vegan: Community Ecology Package. R package version 2.4-2. Available from: www.r-project.org.

Olden J. D. and Kennard M. J. (2010). Intercontinental comparison of fish life history strategies along a gradient of hydrologic variability. *American Fisheries Society Symposium, 73,* 83–107.

Oliva-Paterna F. J., Torralva M. and Fernández-Delgado C. (2006). Threatened fishes of the world: *Aphanius iberus* (Cuvier & Valenciennes, 1846) (Cyprinodontidae). *Environmental Biology of Fishes, 75*, 307–309.

Ormerod S. J., Dobson M., Hildrew A. G. and Townsend C. R. (2010). Multiple stressors in freshwater ecosystems. *Freshwater Biology, 55*, 1–4.

Otto R. G. (2006). Temperature tolerance of the mosquitofish, *Gambusia affinis* (Baird and Girard). *Journal of Fish Biology, 5,* 575–585.

Oufiero C. E. and Whitlow K. R. (2016). The evolution of phenotypic plasticity in fish swimming. *Current Zoology, 62,* 475–488.

Oufiero C. E. and Garland T. (2007). Evaluating performance costs of sexually selected traits. *Functional Ecology, 21*, 676–689.

P

Palmer M. and Ruhi A. (2019). Linkages between flow regime, biota, and ecosystem processes: Implications for river restoration. *Science, 365,* eaaw2087.

Pang X., Shao F., Ding S. H., Fu S. J. and Zhang Y. G. (2019). Interspecific differences and ecological correlations of energy metabolism traits in freshwater fishes. *Functional Ecology, 34,* 616–630.

Pang X., Fu S. J. and Zhang Y. G. (2015). Individual variation in metabolism and swimming performance in juvenile black carp *Mylopharyngodon piceus* and the effects of hypoxia. *Marine and Freshwater Behaviour and Physiology, 48,* 431–443.

Patrick C. J. and Yuan L. L. (2017). Modeled hydrologic metrics show links between hydrology and the functional composition of stream assemblages: *Ecological Applications, 27,* 1605–1617.

Peake S., McKinley R. S. and Scruton D.A. (1997). Swimming performance of various freshwater Newfoundland salmonids relative to habitat selection and fishway design. *Journal of Fish Biology, 51*, 710–723.

Pereda O., von Schiller D., García-Baquero G., Mor J. R., Acuña V., et al. (2021). Combined effects of urban pollution and hydrological stress on ecosystem functions of Mediterranean streams. *Science of the Total Environment, 753,* 141971.

Pettersen A. K., Marshall D. J. and White C. R. (2018). Understanding variation in metabolic rate. *Journal of Experimental Biology, 221,* jeb166876.

Peterson B. J., Wollheim W. M., Mulholland P. J., Webster J. R., Meyer J. L., et al. (2001). Control of nitrogen export from headwaters by headwater streams. *Science, 292*, 86–90.

Pettersson L. B. and Bronmark C. (1999). Energetic consequences of an inducible morphological defence in crucian carp. *Oecologia, 121*, 12–18.

Pettersson L. B. and Bronmark C. (1997). Density-dependent costs of an inducible morphological defense in crucian carp. *Ecology, 78*, 1805–1815.

Pettersson L. B. and Hedenström A. (2000). Energetics, cost reduction and functional consequences of fish morphology. *Proceedings of the Royal Society B: Biological Sciences, 267*, 759–764.

Petrovic M., Ginebreda A., Acuña V., Batalla R. J., Elosegi A., et al. (2011). Combined scenarios of chemical and ecological quality under water scarcity in Mediterranean rivers. *TrAC Trends in Analytical Chemistry, 30*, 1269–1278.

Phillips G., Kelly M., Teixeira H., Salas F., Free G., et al. (2018). Best practice for establishing nutrient concentrations to support good ecological status. Technical Report EUR 29329 EN. Publications Office of the European Union, Luxembourg.

Plaut I. (2002). Does pregnancy affect swimming performance of female Mosquitofish, *Gambusia affinis*? *Functional Ecology, 16*, 290–295.

Plaut I. (2001). Critical swimming speed: Its ecophysiological relevance. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 126*, 121.

Plaut I. (2000). Resting metabolic rate, critical swimming speed, and routine activity of the euryhaline cyprinodontid, *Aphanius dispar*, acclimated to a wide range of salinities. *Physiological and Biochemical Zoology, 73*, 590–596.

Poff N. L. R. and Allan J. D. (1995). Functional Organization of Stream Fish Assemblages in Relation to Hydrological Variability. *Ecology, 76*, 606–627.

Poff N. L. R., Allan J. D., Bain M. B., Karr J. R., Prestegaard K. L., et al. (1997). The natural flow regime: A paradigm for river conservation and restoration. *BioScience, 47*, 769–784.

Polis G. A., Sears A. L. W., Huxel G. R., Strong D. R. and Maron J. (2000). When is a trophic cascade a trophic cascade? *Trends in Ecology & Evolution, 15*, 473–475.

Ponsatí L., Corcoll N., Petrović M., Picó Y., Ginebreda A., et al. (2016). Multiple-stressor effects on river biofilms under different hydrological conditions. *Freshwater Biology, 61*, 2102–2115.

Pörtner H. O. and Knust R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science, 315*, 95–97

Power M., Lowe R., Furey P., Welter J., Limm M., et al. (2009). Algal mats and insect emergence in rivers under Mediterranean climates: towards photogrammetric surveillance. *Freshwater Biology, 54*, 2101–2115.

Power M. E. (1992). Hydrologic and trophic controls of seasonal algal blooms in northern California rivers. *Archiv Fur Hydrobiologie, 125*, 385–410.

Power M. E. (1990). Resource enhancement by indirect effects of grazers: Armored catfish, algae, and sediment. *Ecology, 71*, 897–904.

Price K. J. and Carrick H. J. (2016). Effects of experimental nutrient loading on phosphorus uptake by biofilms: evidence for nutrient saturation in mid-Atlantic streams. *Freshwater Science, 35*, 503–517.

Proia L., Romaní A. and Sabater S. (2017). Biofilm phosphorus uptake capacity as a tool for the assessment of pollutant effects in river ecosystems. *Ecotoxicology, 26*, 271–282.

Propst D. L. and Gido K. B. (2004). Responses of Native and Nonnative Fishes to Natural Flow Regime Mimicry in the San Juan River. *Transactions of the American Fisheries Society, 133*, 922–931.

Q

Qu X., Wu N., Tang T., Cai Q. and Park Y-S. (2010) Effects of heavy metals on benthic macroinvertebrate communities in high mountain streams. *Annales de Limnologie - International Journal of Limnology, 46*, 291–302.

R

R Development Core Team. (2018). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. Available from: www.r-project.org.

Reardon J., Foreman J. A. and Searcy R. L. (1966). New reactants for the colorimetric determination of ammonia. *Clinica Chimica Acta, 14,* 403–405.

Reid G. M., Contreras MacBeath T. and Csatádi K. (2013). Freshwater Fishes and Their Conservation: Global Challenges. *International Zoo Yearbook, 47,* 6-45.

Reidy S. P., Kerr S. R. and Nelson J. A. (2000). Aerobic and anaerobic swimming performance of individual Atlantic cod. *Journal of Experimental Biology, 203,* 347–357.

Resh V. H., Brown A. V., Covich A. P., Gurtz M. E., Li H. W., et al. (1988). The Role of Disturbance in Stream. *Ecology Journal of the North American Benthological Society, 7,* 433–455.

Ribot M., von Schiller D. and Martí E. (2017). Understanding pathways of dissimilatory and assimilatory dissolved inorganic nitrogen uptake in streams. *Limnology and Oceanography, 62,* 1166–1183.

Riley R. H., Townsend C. R., Raffaelli D. A. and Flecker A. S. (2004). Sources and effects of subsidies along the stream-estuary continuum. In: Polis G.A., Power M.E. and Huxel G.R. (Eds.), *Food Webs at the Landscape Level*. Chicago, U.S.: University of Chicago Press.

Ripple W. J., Estes J. A., Schmitz O. J., Self K. E., Taylor D. S. and Wolf C. (2016). What is a Trophic Cascade? *Trends in Ecology & Evolution, 31,* 842–849.

Rincón P. A., Correas A. M., Morcillo F., Risueño P. and Lobón-Cerviá J. (2002). Interaction between the introduced eastern mosquitofish and two autochthonous Spanish toothcarps. *Journal of Fish Biology, 61*, 1560–1585.

Robinson C. T., Schuwirth N., Baumgartner S. and Stamm C. (2014). Spatial relationships between land-use, habitat, water quality and lotic macroinvertebrates in two Swiss catchments. *Aquatic Sciences, 76*, 375–392.

Rodrigues A. C. M., Machado A. L., Bordalo M. D., Saro L., Simão F. C. P., et al. (2018). Invasive Species Mediate Insecticide Effects on Community and Ecosystem Functioning. *Environmental Science & Technology, 52*, 4889–4900.

Rodríguez-Lozano P., Rieradevall M. and Prat N. (2016a). Top predator absence enhances leaf breakdown in an intermittent stream. *Science of the Total Environment, 572*, 1123–1131.

Rodríguez-Lozano P., Verkaik I., Maceda-Veiga A., Monroy M., de Sostoa, A., et al. (2016b). A trait-based approach reveals the feeding selectivity of a small endangered Mediterranean fish. *Ecology and Evolution, 6*, 3299–3310.

Rodríguez-Lozano P., Verkaik I., Rieradevall M. and Prat N. (2015). Small but powerful: top predator local extinction affects ecosystem structure and function in an intermittent stream. *PLoS One, 10*, e0117630.

Rohlf F. J. (2015). The tps series of software. *Hystrix 26*, 9–12.

Romaní A. M., Artigas J. and Ylla I. (2012). Extracellular enzymes in aquatic biofilms: microbial interactions vs water quality effects in the use of organic matter. In: Lear G. and Lewis G. (Eds.), *Microbial Biofilms: Current Research and Applications*. UK: Caister Academic Press.

Romero F., Acuña V., Font C., Freixa A. and Sabater S. (2019). Effects of multiple stressors on river biofilms depend on the time scale. *Scientific Reports, 9*, 1–12.

Rosenberg D. M., McCully P. and Pringle C. M. (2000). Global-scale environmental effects of hydrological alterations: introduction. *Bioscience, 50*, 746–751.

Rosenfeld J. (2000). Effects of fish predation in erosional and depositional habitats in a temperate stream. *Canadian Journal of Fisheries and Aquatic Sciences, 57*, 1369–1379.

Royle N. J., Metcalfe N. B. and Lindström J. (2006). Sexual selection, growth compensation and fast-start swimming performance in Green Swordtails, *Xiphophorus helleri*. *Functional Ecology, 20*, 662–669.

Rubio-Gracia F., García-Berthou E., Latorre D., Moreno-Amich R., Srean P., et al. (2020). Differences in swimming performance and energetic costs between an endangered native toothcarp (*Aphanius iberus*) and an invasive mosquitofish (*Gambusia holbrooki*). *Ecology of Freshwater Fish, 29*, 230–240.

Rubio-Gracia F., Almeida D., Bonet B., Casals F., Espinosa C., et al. (2017). Combined effects of hydrologic alteration and cyprinid fish in mediating biogeochemical processes in a Mediterranean stream. *Science of the Total Environment, 601–602*, 1217–1225.

Ruhí A., Muñoz I., Tornés E., Batalla R. J., Vericat D., et al. (2016). Flow regulation increases food-chain length through omnivory mechanisms in a Mediterranean river network. *Freshwater Biology, 61*, 1536–1549.

S

Saaristo M., Brodin T., Balshine S., Bertram M. G., Brooks B. W., et al. (2018). Direct and indirect effects of chemical contaminants on the behaviour, ecology and evolution of wildlife. *Proceedings of the Royal Society B: Biological Sciences, 285*, 20181297.

Sabater S., Navarro E. and Guasch H. (2002a). Effects of copper on algal communities at different current velocities. *Journal of Applied Phycology, 14*, 391–398.

Sabater S., Guasch H., Romaní A. and Muñoz I. (2002b). The effect of biological factors on the efficiency of river biofilms in improving water quality. *Hydrobiologia, 469,* 149–156.

Sabater, S., Bregoli, F., Acuña, V. Barceló D., Elosegi A., et al. (2018). Effects of human-driven water stress on river ecosystems: a meta-analysis. *Scientific Reports, 8,* 11462.

Sabater S., Barceló D., De Castro-Català N., Ginebreda A., Kuzmanovic M., et al. (2016). Shared effects of organic microcontaminants and environmental stressors on biofilms and invertebrates in impaired rivers. *Environmental Pollution, 210*, 303–314.

Santoro A., Blo G., Mastrolitti S. and Fagioli F. (2009). Bioaccumulation of heavy metals by aquatic macroinvertebrates along the Basento River in the South of Italy. *Water, Air, & Soil Pollution, 201*, 19–31.

Scarnecchia D. L. (1988). The importance of streamlining in influencing fish community structure in channelized and unchannelized reaches of a prairie stream. *Regulated Rivers: Research & Management, 2,* 155–166.

von Schiller D., Martí E., Riera J. L. and Sabater F. (2007). Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses. *Freshwater Biology, 52*, 891–906.

Schindler D. W. (1998). Replication versus Realism: The Need for Ecosystem-Scale Experiments. *Ecosystems, 1,* 323–334.

Schinegger R., Palt M., Segurado P. and Schmutz S. (2016). Untangling the effects of multiple human stressors and their impacts on fish assemblages in European running waters. *Science of the Total Environment, 573,* 1079–1088.

Schulte P. M. (2015). The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *Journal of Experimental Biology, 218*, 1856–1866.

Schmidt-Nielsen K. (1972). Locomotion: energy cost of swimming, flying and running. *Science, 177*, 222–228.

Schmitz O. J. (2008). From Mesocosms to the Field: The Role and Value of Cage Experiments in Understanding Top-Down Effects in Ecosystems. In: Weisser W. W. and Siemann E. (Eds.), *Insects and Ecosystem Function. Ecological Studies (Analysis and Synthesis)*. Vol 173. Berlin, Heidelberg: Springer.

Schmitz O. J., Krivan V. and Ovadia O. (2004). Trophic cascades: The primacy of trait-mediated indirect interactions. *Ecology Letters, 7*, 153–163.

Schneider F. D. and Brose U. (2013). Beyond diversity: how nested predator effects control ecosystem functions. *Journal of Animal Ecology, 82*, 64–71.

Secor S. M. (2009). Specific dynamic action: a review of the postprandial metabolic response. *Journal of Comparative Physiology B, 179*, 1–56.

Segner H., Schmitt-Jansen M. and Sabater S. (2014). Assessing the impact of multiple stressors on aquatic biota: The receptor's side matters. *Environmental Science & Technology, 48*, 7690–7696.

Shivoga W. A. (2001). The influence of hydrology on the structure of invertebrate communities in two streams flowing into Lake Nakuru, Kenya. *Hydrobiologia, 458*, 121–130.

Silva S. S., Alexandre C. M., Quintella B. R. and de Almeida P. R. (2021). Seasonal environmental variability drives the swimming performance of a resident Iberian fish. *Ecology of Freshwater Fish, 30,* 366–374.

Smith H., Wood P. J. and Gunn J. (2003). The influence of habitat structure and flow permanence on invertebrate communities in karst spring systems. *Hydrobiologia, 510,* 53–66.

Smolders A. J. P., Lock R. A. C., Van der Velde G., Medina Hoyos R. I. and Roelofs J. G. M. (2003). Effects of mining activities on heavy metal concentrations in water, sediment, and macroinvertebrates in different reaches of the Pilcomayo River, South America. *Archives of Environmental Contamination and Toxicology, 44*, 314–323.

Solà C., Burgos M., Plazuelo Á., Toja J., Plans M. and Prat N. (2004). Heavy metal bioaccumulation and macroinvertebrate community changes in a Mediterranean stream affected by acid mine drainage and an accidental spill (Guadiamar River, SW Spain). *Science of the Total Environment, 333*, 109–126.

Spooner D. E., Frost P. C., Hillebrand H., Arts M. T., Puckrin O. and Xenopoulos M. A. (2013). Nutrient loading associated with agriculture land use dampens the importance of consumer-mediated niche construction. *Ecology Letters, 16*, 1115–1125.

Spranza J. J. and Stanley E. H. (2000). Condition, growth, and reproductive styles of fishes exposed to different environmental regimes in a prairie drainage. *Environtal Biology of Fishes, 59*, 99–109.

Srean P., Almeida D., Rubio-Gracia F., Luo Y. and García-Berthou E. (2016). Effects of size and sex on swimming performance and metabolism of invasive mosquitofish *Gambusia holbrooki*. *Ecology of Freshwater Fish, 26*, 424–433.

Steinman A. D., Lamberti G. A. and Leavitt P. R. (2007). Biomass and pigments of benthic algae. In: Hauer, F. R., and Lamberti, G. A. (Eds.), *Methods in Stream Ecology*. London, UK: Academic press.

Stelzer R. S. and Lamberti G. A. (2002). Ecological stoichiometry in running waters: Periphyton chemical composition and snail growth. *Ecology, 83*, 1039–1051.

Stoffels R. J. (2015). Physiological trade-offs along a fast-slow lifestyle continuum in fishes: What do they tell us about resistance and resilience to hypoxia? *PLoS ONE, 10*, 1–19.

Stone L. and Weisburd R. S. J. (1992). Positive feedback in aquatic ecosystems. *Trends in Ecology & Evolution, 7*, 263–267.

Svendsen J. C., Banet A. I., Christensen R. H. B., Steffensen J. F. and Aarestrup K. (2013). Effects of intraspecific variation in reproductive traits, pectoral fin use and burst swimming on metabolic rates and swimming performance in the Trinidadian guppy (*Poecilia reticulata*). *Journal of Experimental Biology, 216,* 3564–3574.

Svendsen J. C., Tudorache C., Jordan A. D., Steffensen J. F., Aarestrup K. and Domenici P. (2010). Partition of aerobic and anaerobic swimming costs related to gait transitions in a labriform swimmer. *Journal of Experimental Biology, 213,* 2177–2183.

T

Tachet H., Richoux P., Bournard M. and Usseglio-Polatera P. (2000). Invertébrés d'eau douce: systématique, biologie, écologie. Paris, France: CNRS éditions.

Taylor J. M., Vanni M. J. and Flecker A. S. (2015). Top-down and Bottom-up interactions in Freshwater Ecosystems: Emerging Complexities. In: Hanley T.C. and La Pierre K.J. (Eds.), *Trophic ecology: bottom-up and top-down interactions across aquatic and terrestrial systems*. Cambridge, UK: Cambridge University Press.

Tedesco P., Beauchard O., Bigorne R., Blanchet S., Buisson L., et al. (2017). A global database on freshwater fish species occurrence in drainage basins. *Scientific Data, 4*, 170141.

Thiem J. D., Dawson J. W., Hatin D., Danylchuk A. J, Dumont P., et al. (2016). Swimming activity and energetic costs of adult lake sturgeon during fishway passage. *Journal of Experimental Biology, 219,* 2534–2544.

Tierney K. B., Kasurak A. V., Zielinski B. S. and Higgs D. M. (2011). Swimming performance and invasion potential of the round goby. *Environmental Biology of Fishes, 92,* 491–502.

Tikkanen P., Muotka T. and Huhta A. (1994). Predator detection and avoidance by lotic mayfly nymphs of different size. *Oecologia, 99,* 252–259.

Timoner X., Buchaca T., Acuña V. and Sabater S. (2014). Photosynthetic pigment changes and adaptations in biofilms in response to flow intermittency. *Aquatic Sciences, 76*, 565–578.

Tlili A., Corcoll N., Bonet B., Morin S., Montuelle B., et al. (2011). In situ spatio-temporal changes in pollution-induced community tolerance to zinc in autotrophic and heterotrophic biofilm communities. *Ecotoxicology, 20,* 1823–1839.

Townsend C. R., Uhlmann S. S. and Matthaei C. D. (2008). Individual and combined responses of stream ecosystems to multiple stressors. *Journal of Applied Ecology, 45*, 1810–1819.

Truchy A., Angeler D. G., Sponseller R. A., Johnson R. K. and McKie B. G. (2015). Linking biodiversity, ecosystem functioning and services, and ecological resilience: Towards an integrative framework for improved management. *Advances in Ecological Research, 53,* 55–96.

Tucker V. A. (1970). Energetic cost of locomotion in animals. *Comparative Biochemistry and Physiology, 34*, 841–846.

Tudorache C., Viaene P., Blust R., Vereecken H. and De Boeck G. (2008). A comparison of swimming capacity and energy use in seven European freshwater fish species. *Ecology of Freshwater Fish, 17,* 284–291.

V

Van den Brink P. J., Choung C. B., Landis W., Mayer-Pinto M., Pettigrove V., et al. (2016). New approaches to the ecological risk assessment of multiple stressors. *Marine and Freshwater Research, 67*, 429–439.

Vanni M. J. (2010). When and Where Do Fish Have Strong Effects on Stream Ecosystem Processes. *American Fisheries Society Symposium, 73*, 531–538.

Vanni M. J. (2002). Nutrient cycling by animals in freshwater ecosystems. *Annual Review of Ecology and Systematics, 33*, 341–370.

Vannote R. L., Inshall G. W. A. M., Ummins K. E. W. C., Edell J. A. R. S. and Ushing C. O. E. C. (1980). Pengantar Limnologi. *Canadian Journal of Fisheries and Aquatic Sciences, 37*, 130–137.

Vargas-Amelin E. and Pindado P. (2014). The challenge of climate change in Spain: Water resources, agriculture and land. *Journal of Hydrology, 518*, 243–249.

Videler J. J. (1993). Fish swimming. Fish and fisheries series (1st edition). London, UK: Chapman and Hall.

Vila-Gispert A., Alcaraz C. and García-Berthou E. (2005). Life-history traits of invasive fish in small Mediterranean streams. *Biological Invasions, 7,* 107–116.

Vinebrooke R. D., Cottingham K. L., Norberg J., Scheffer M., Dodson S. I., et al. (2004). Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. *Oikos, 104,* 451–457.

Vinyoles D., Robalo J. I., de Sostoa A., Almodóvar, A., Elvira B., et al. (2007). Spread of the alien bleak *Alburnus alburnus* (Linnaeus, 1758) (Actinopterygii, Cyprinidae) in the Iberian Peninsula: The role of reservoirs. *Graellsia, 63,* 101–110.

W

Wainwright P. C., Bellwood D. R. and Westneat M. W. (2002). Ecomorphology of locomotion in labrid fishes. *Environmental Biology of Fishes, 65*, 47–62.

Wallace J. B. and Webster J. R. (1996). The role of macroinvertebrates in stream ecosystem function. *Annual Review of Entomology, 41*, 115–139.

Walker J. A., Alfaro M. E., Noble M. M. and Fulton C. J. (2013). Body fineness ratio as a predictor of maximum prolonged-swimming speed in coral reef fishes. *PLoS ONE, 8*, e75422.

Ward D. L., Schultz A. A. and Matson P. G. (2003). Differences in swimming ability and behavior in response to high water velocities among native and nonnative fishes. *Environmental Biology of Fishes, 68*, 87–92.

Ward J. V. (1989). The Four-Dimensional Nature of Lotic Ecosystems. *Journal of the North American Benthological Society, 8*, 2–8.

Webb P. W. (1994). The biology of fish swimming. In Maddock L., Bone Q. and Rayner J. M. V. (Eds.), *The Mechanics and physiology of animal swimming*. Cambridge, UK: Cambridge University Press.

Webb P. W. (1984). Body form, locomotion and foraging in aquatic vertebrates. *Integrative and Comparative Biology, 24*, 107–120.

Webb P. W. (1975). Hydrodynamics and energetics of fish propulsion. *Bulletin of the Fisheries Research Board of Canada, 190*, 1–158.

Webb P. W. and Weihs D. (1986). Functional locomotor morphology of early life history stages of fishes. *Transactions of the American Fisheries Society, 115*, 115–127.

Wernersson A-S., Carere M., Maggi C., Tusil P., Soldan P., et al. (2015). The European technical report on aquatic effect-based monitoring tools under the water framework directive. *Environmental Sciences Europe, 27*, 7.

Wesner J. S. (2013). Fish predation alters benthic, but not emerging, insects across whole pools of an intermittent stream. *Freshwater Science, 32*, 438–449.

Williams L. R., Taylor C. M. and Warren M. L. (2003). Influence of fish predation on assemblage structure of macroinvertebrates in an intermittent stream. *Transactions of the American Fisheries Society, 132*, 120–130.

White C. R., Marshall D. J., Alton L. A., Arnold P. A., Beaman J. E., et al. (2019). The origin and maintenance of metabolic allometry in animals. *Nature Ecology & Evolution, 3*, 598–603.

White S. J., Tells T. J. and Wilson A. J. (2016). Metabolism, personality and pace of life in the Trinidadian guppy, *Poecilia reticulate. Behaviour, 153*, 1517–1543.

Wilson R. S. (2005). Temperature influences the coercive mating and swimming performance of male eastern mosquitofish. *Animal Behaviour, 70*, 1387–1394.

Winemiller K. O. and Jepsen D. B. (2004). Migratory neotropical fishes subsidize food webs of oligotrophic blackwater rivers. In: Polis G. A., Power M. E. and Huxel G. R. (Eds.), *Food Webs at the Landscape Leve*l. Chicago: University of Chicago Press.

Winemiller K. O., Montaña C. G., Roelke D. L., Cotner J. B., Montoya J. V., et al. (2014). Pulsing hydrology determines top-down control of basal resources in a tropical river-floodplain ecosystem. *Ecological Monographs, 84*, 621–635.

Winkelmann C., Schneider J., Mewes D., Schmidt S. I., Worischka S., et al. (2014). Top-down and bottom-up control of periphyton by benthivorous fish and light supply in two streams. *Freshwater Biology, 59*, 803–818.

Wootton J. T. (1994). The Nature and Consequences of Indirect Effects in Ecological Communities. *Annual Review of Ecology, Evolution, and Systematics, 25*, 443–466.

Y

Yan G. J., He X. K., Cao Z. D. and Fu S. J. (2013). An interspecific comparison between morphology and swimming performance in cyprinids. *Journal of Evolutionary Biology, 26*, 1802–1815.

Z

Zeiringer B., Seliger C., Greimel F. and Schmutz S. (2018). River Hydrology, Flow Alteration, and Environmental Flow. In: Schmutz S. and Sendzimir J. (Eds.), *Riverine Ecosystem Management. Aquatic Ecology Series*. Vol 8. Cham, Germany: Springer.

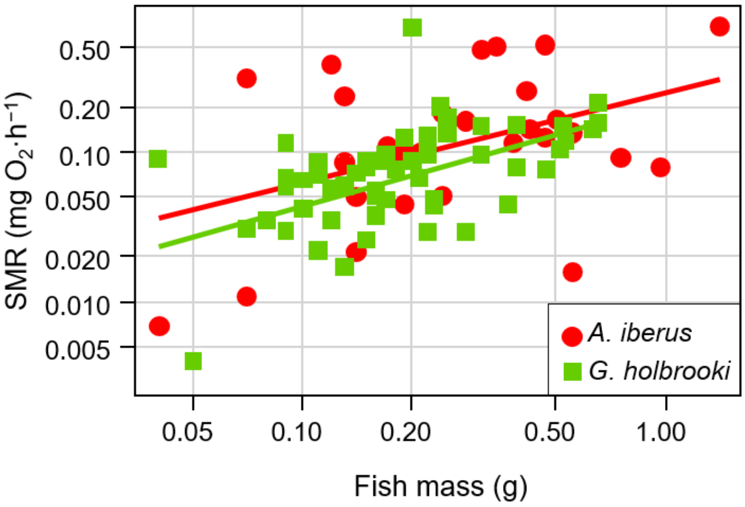
Zhang Y., Huang Q., Liu S., Dingcong H., Weim G., et al. (2014). Intraspecific mass scaling of metabolic rates in grass scarp. *Journal of Comparative Physiology B, 184*, 347–354.

Zou K., Thébault E., Lacroix G. and Barot S. (2016). Interactions between the green and brown food web determine ecosystem functioning. *Functional Ecology, 30*, 1454–1465.

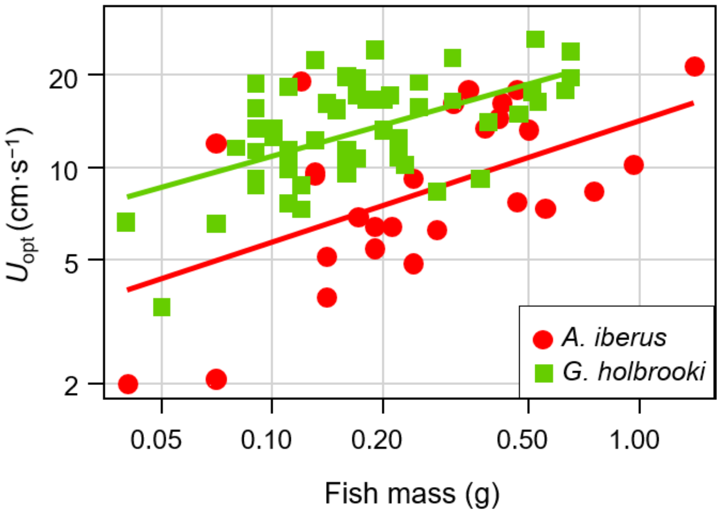
8. SUPPLEMENTARY MATERIAL

Supplementary materials for Chapter I:

Differences in swimming performance and energetic costs between an endangered native toothcarp (*Aphanius iberus*) and an invasive mosquitofish (*Gambusia holbrooki*)



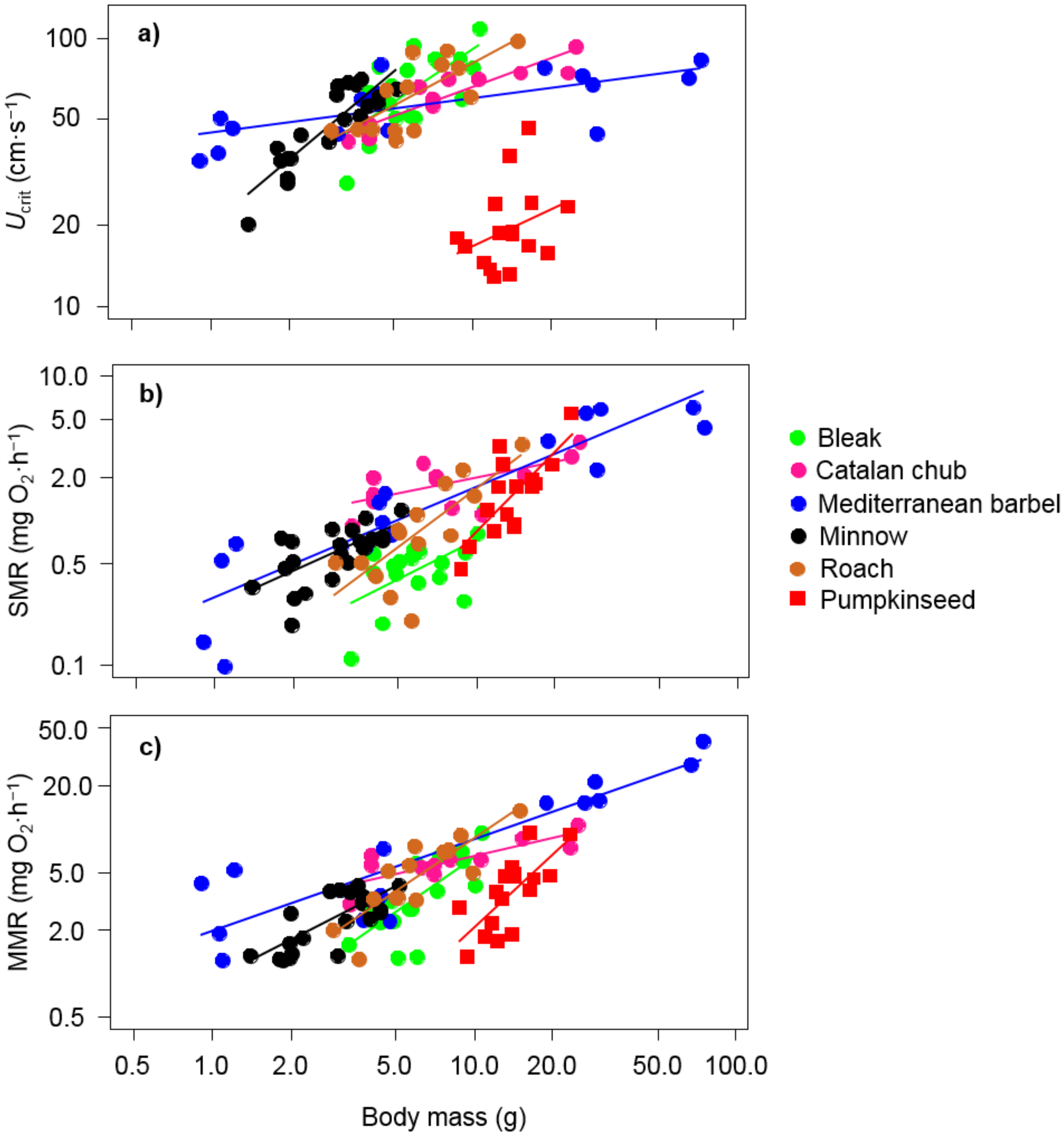
F I G U R E S1 Relationship of standard metabolic rate SMR with fish mass for Spanish toothcarp, *Aphanius iberus* (N = 28) and mosquitofish, *Gambusia holbrooki* (N = 60). Regressions are not significantly different between species for SMR (Table 1), and thus a single linear regression is shown by species. Regression coefficients are shown in Table 2. Note both axes are on a log scale



F I G U R E S2 Relationship of optimal swimming speed U_{opt} with fish mass for Spanish toothcarp, *Aphanius iberus* (N = 28) and mosquitofish, *Gambusia holbrooki* (N = 60). The assumption of parallelism was not satisfied for U_{opt}, and therefore ANCOVA was not applied. Regression coefficients are shown in Table 2. Note both axes are on a log scale

Supplementary materials for Chapter II:

Size-related effects and the influence of metabolic traits and morphology on swimming performance in fish



F I G U R E S1 Relationships of critical swimming speed (U_{crit}) (a), standard metabolic rate (SMR) (b) and maximal metabolic rate (MMR) (c) with body mass for bleak, Catalan chub, Mediterranean barbel, minnow, roach, and pumpkinseed. Cyprinid species are represented by circles. Linear regressions functions are shown in Table 1. Both axes are on a log scale

TABLE S1 Linear regression functions ($\log\dot{M}_{O_2}$ = log *a* + *bU*) estimated through the exponential functions shown in Figure 1 (Chapter II)

	N	Mass range	Mean ± SD	log*a*	95% CI	*b*	95% CI	R^2
Bleak	6	0–5 g	4.23 ± 0.60 g	-0.76**	(-1.16, -0.76)	0.03***	(0.03, 0.03)	0.47
	9	5–10 g	6.77 ± 1.46 g	-1.24***		0.03***		0.72
	2	10–15 g	10.32 ± 0.45 g	-0.09		0.02***		0.85
Catalan chub	4	0–5 g	3.84 ± 0.33 g	0.35**	(0.49, 0.76)	0.03***	(0.01, 0.02)	0.70
	4	5–10 g	7.09 ± 0.76 g	0.64***		0.02***		0.39
	2	10–15 g	12.88 ± 3.36 g	0.74***		0.02***		0.91
	2	20–25 g	23.99 ± 0.86 g	1.08***		0.01***		0.94
Mediterranean barbel	10	0–5 g	2.88 ± 1.63 g	-0.55***	(-0.38, 0.28)	0.03***	(0.02, 0.04)	0.48
	1	19 g	18.84 g	1.15**		0.02**		0.80
	3	25–30	28.32 ± 1.95 g	2.16***		0.01***		0.75
	2	65–75 g	71.17 ± 4.18 g	1.96***		0.01**		0.59
Minnow	21	0–6 g	2.96 ± 1.03 g	-0.47***	(-0.58, -0.36)	0.02***	(0.02, 0.03)	0.58
Roach	5	0–5 g	4.05 ± 0.84 g	-0.68***	(-0.48, -0.14)	0.03***	(0.02, 0.03)	0.64
	8	5–10 g	7.09 ± 1.72 g	-0.13		0.02***		0.71
	1	15 g	14.9 g	1.21***		0.01***		0.94

Pumpkinseed	2	5–10 g	9.12 ± 0.48 g	-0.65**	(-0.09, 0.41)	0.07***	(0.03, 0.06)	0.94
	10	10–15 g	12.96 ± 1.16 g	0.05		0.04***		0.33
	4	15–20 g	17.35 ± 1.45 g	0.61***		0.03***		0.91
	1	23 g	23.32 g	1.53*		0.03*		0.99
Spanish toothcarp	41	0–0.9 g	0.34 ± 0.14 g	-2.60***	(-2.71, -2.46)	0.09***	(0.08, 0.11)	0.42
Mosquitofish	42	0–0.3 g	0.15 ± 0.06 g	-2.87***	(-2.84, -2.67)	0.08***	(0.07, 0.09)	0.39
	8	0.3–0.6 g	0.42 ± 0.09 g	-2.20***		0.06***		0.56
	3	0.6–0.9 g	0.64 ± 0.01 g	-1.94***		0.05***		0.81

CI, 95% confidence intervals for regression coefficients. Note: A single 95% CI is given for each species (i.e. including all fish). A single regression function is shown for minnow and Spanish toothcarp because the mass-range groups were not statistically different (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$)

Supplementary materials for Chapter IV:

Indirect food-web interactions are mediated by metal pollution and an ecosystem engineer, *Barbus meridionalis* (Cyprinidae), in a Mediterranean stream

TABLE S1 Linear relationships of trace metal concentration (µg g^{-1}) between four macroinvertebrate families and periphyton (in µg g^{-1})

	Stonefly (Leuctridae)				Caddisfly (Hydropsychidae)				Snail (Lymnaeidae)				Dragonfly (Gomphidae)			
	Estimate b ± SE	*F*	*P*	R^2	Estimate b ± SE	*F*	*P*	R^2	Estimate b ± SE	*F*	*P*	R^2	Estimate b ± SE	*F*	*P*	R^2
Zn	0.35 ± 0.07	26.43	**0.014**	0.90	0.30 ± 0.03	98.52	**0.002**	0.97	0.27 ± 0.07	14.76	0.162	0.94	0.25 ± 0.02	106.30	**0.002**	0.97
Mn	0.35 ± 0.13	7.21	0.075	0.71	0.04 ± 0.07	0.3	0.619	0.09	0.04 ± 0.01	8.37	0.212	0.89	0.34 ± 0.12	8.27	0.064	0.73
Fe	0.26 ± 0.09	8.67	0.06	0.66	0.29 ± 0.20	2.07	0.246	0.41	0.29 ± 0.28	1.05	0.492	0.54	0.36 ± 0.17	4.61	0.121	0.61
Pb	0.29 ± 0.03	72.96	**0.003**	0.96	0.32 ± 0.04	80.53	**0.003**	0.96	0.17 ± 0.05	12.62	0.175	0.93	0.25 ± 0.02	136.30	**0.001**	0.98
Ni	0.20 ± 0.13	2.48	0.213	0.45	0.62 ± 0.37	2.76	0.195	0.48	0.14 ± 0.48	0.08	0.821	0.08	-0.23 ± 0.32	0.51	0.526	0.15
Cr	0.12 ± 0.20	0.36	0.593	0.11	0.15 ± 0.22	0.43	0.557	0.13	0.32 ± 0.02	413.3	**0.03**	0.99	0.43 ± 0.09	24.49	**0.016**	0.89
Cd	0.43 ± 0.08	26.28	**0.014**	0.90	0.36 ± 0.08	21.45	**0.019**	0.88	0.34 ± 0.39	0.78	0.539	0.44	0.22 ± 0.05	18.0	**0.02**	0.86
Cu	0.39 ± 0.48	0.68	0.471	0.18	0.25 ± 0.30	0.71	0.461	0.19	0.45 ± 0.36	1.52	0.433	0.60	0.04 ± 0.55	0.01	0.942	0.002

The degrees of freedom are 3 for Leuctridae, Hydropsychidae and Gomphidae and 1 for Lymnaeidae. Variables were square root transformed. Significant results ($P < 0.05$) are in boldface.