UNIVERSITAT DE
BARCELONA

Fire legacies in Mediterranean streams: the key roles of the riparian canopy and the top predator on food webs

Pablo Rodríguez Lozano

Aquesta tesi doctoral està subjecta a la llicència ***Reconeixement- NoComercial – CompartirIgual 4.0. Espanya de Creative Commons***.

Esta tesis doctoral está sujeta a la licencia ***Reconocimiento - NoComercial – CompartirIgual 4.0. España de Creative Commons.***

This doctoral thesis is licensed under the ***Creative Commons Attribution-NonCommercial-ShareAlike 4.0. Spain License.***

Pablo Rodríguez Lozano

Fire legacies itn Mediterranean streams:
the key roles of the riparian canopy and the top predator on food webs

Fire legacies in Mediterranean streams: the key roles of the riparian canopy and the top predator on food webs

Pablo Rodríguez Lozano

UNIVERSITAT DE BARCELONA

Fire legacies in Mediterranean streams: the key roles of the riparian canopy and the top predator on food webs

Pablo Rodríguez Lozano

Pablo Rodríguez Lozano. Fire legacies in Mediterranean streams: the key roles of the riparian canopy and the top predator on food webs. PhD thesis. Universidad de Barcelona, Barcelona.

Cover: Untitled, oil on canvas, 61 x 46 cm, 2015. By Javier Rodríguez Lozano.

Covers of Chapter 1, Chapter 2, Chapter 3, and Chapter 4: Untitled, oil on canvas, 35 x 38 cm, 2015. Each one. By Javier Rodríguez Lozano.

TESIS DOCTORAL

Universidad de Barcelona
Departamento de Ecología
Programa de Doctorado: Ecología Fundamental y Aplicada

Fire legacies in Mediterranean streams: the key roles of the riparian canopy and the top predator on food webs

Legados del fuego en ríos mediterráneos: el papel clave de la cobertura riparia y del superdepredador en las redes tróficas

Memoria presentada por Pablo Rodríguez Lozano para optar al título de
Doctor por la Universidad de Barcelona

Pablo Rodríguez Lozano
Barcelona, diciembre 2015

Aprobación de los directores de la tesis doctoral:

In memoriam

Dr. Narcís Prat Fornells
Catedrático de Ecología
Universidad de Barcelona

Dra. Maria Rieradevall Sant
Profesora Titular
Universidad de Barcelona

A los ríos

—¡Buenos días! —dijo.
Era un jardín cuajado de rosas.
—¡Buenos días! —dijeron las rosas.
El principito las miró. ¡Todas se parecían tanto a su flor!
—¿Quiénes son ustedes? —les preguntó estupefacto.
—Somos las rosas —respondieron éstas.
—¡Ah! —exclamó el principito.
Y se sintió muy desgraciado. Su flor le había dicho que era la única de su especie en todo el universo. ¡Y ahora tenía ante sus ojos más de cinco mil todas semejantes, en un solo jardín […]!
Y luego continuó diciéndose: "Me creía rico con una flor única y resulta que no tengo más que una rosa ordinaria. Eso y mis tres volcanes que apenas me llegan a la rodilla y uno de los cuales acaso esté extinguido para siempre. Realmente no soy un gran príncipe..." Y echándose sobre la hierba, el principito lloró.
Entonces apareció el zorro.
[…]
El principito se fue a ver las rosas a las que dijo:
—No son nada, ni en nada se parecen a mi rosa. Nadie las ha domesticado ni ustedes han domesticado a nadie […]. Son muy bellas, pero están vacías y nadie daría la vida por ustedes. Cualquiera que las vea podrá creer indudablemente que mi rosa es igual que cualquiera de ustedes. Pero ella se sabe más importante que todas, porque yo la he regado, porque ha sido a ella a la que abrigué con el fanal, porque yo le maté los gusanos (salvo dos o tres que se hicieron mariposas) y es a ella a la que yo he oído quejarse, alabarse y algunas veces hasta callarse. Porque es mi rosa, en fin.
Y volvió con el zorro.
—Adiós —le dijo.
—Adiós —dijo el zorro—. He aquí mi secreto, que no puede ser más simple: sólo con el corazón se puede ver bien; lo esencial es invisible para los ojos.
—Lo esencial es invisible para los ojos —repitió el principito para acordarse.
—Lo que hace más importante a tu rosa, es el tiempo que tú has perdido con ella.
—Es el tiempo que yo he perdido con ella... —repitió el principito para recordarlo.
—Los hombres han olvidado esta verdad—dijo el zorro—, pero tú no debes olvidarla. Eres responsable para siempre de lo que has domesticado. Tú eres responsable de tu rosa...

Le Petit Prince
Antoine de Saint-Exupéry

Agradecimientos

Mucha gente me ha ayudado a realizar esta tesis de forma desinteresada o incluso sin ser conscientes de ello. Mil gracias.

En primer lugar, quería dar las gracias a mis directores de tesis, Narcís Prat y Maria Rieradevall, por haber confiado en mí para llevar a cabo la tesis doctoral y por la gran libertad que me han dado. De Narcís me quedo con su amor a los ríos, que está por encima de todo lo demás. De María, con su tenacidad, con su afán de superación. Gracias.

Infinitas gracias a mis dos directores de tesis en la sombra, sin los cuales esto hubiera sido completamente imposible. Muchas gracias Iraima por hacerme heredero, y no solo del FURIMED2. Gracias a ti, Pau, viga maestra. Gracias a los dos por enseñarme tanto y por solucionarme tantísimos problemas. Gracias por todo.

Gracias a los FEM, a los que siguen aquí, Núria B., Pau, Nuria S., Pol, Daniel, Núria C., Raúl, Miguel, y a los que marcharon, Iraima, Blanca, Giorgio, Tano, Rubén, Romain, Eduard, Christian, Caroline, Laura M., Marius, Andrea, Isabelle. Gracias por toda vuestra ayuda en el campo, en lupas, en el lab, con la estadística, en los congresos… Y gracias a Xavi, tú también eres FEM. ¡Qué suerte tuve de caer entre vosotros! ¡Os debo muchas paellas!

Gracias a los ictiólogos. A Albert, Mario y Adolfo, cuya participación ha sido indispensable para llevar a cabo esta tesis. Y a Dolors, por transmitirme este último año su amor a los peces. Gracias también a Lídia, Mary, y Patri por enseñarme los secretos del lab. Gracias a todos con lo que he compartido la sala de lupas, por hacer de ese zulo un espacio tan especial, y a los que manifestaron conmigo la necesidad de mejorarlo.

Durante esta tesis he realizado dos estancias en el extranjero (UK y NZ). Los resultados experimentales de estas estancias no están recogidos en esta tesis, pero las estancias me han servido para aprender muchas cosas que he aplicado en ella. Thanks to Guy Woodward for letting me to collaborate with his research group. Thanks to the London team, especially to Gareth and Eoin. Thanks to Christoph Matthaei for showing me other way of making science. Thanks to Andi, Romana, and to all the people I met in NZ. THANKS.

Gracias a toda la gente que he conocido en los congresos. Por las experiencias vividas en ellos, ¡y por los viajes que me han permitido hacer! Han sido al mismo tiempo un descanso, una escuela, un placer, y un gran empujón para el desarrollo de esta tesis. Gracias de nuevo a mis directores, por animarme a asistir a ellos y por su financiación.

Quería agradecer a todos los doctorandos del departamento de Ecología el compensar con creces todas las cositas negativas de la tesis. A los que estaban acabando la tesis, a los que comenzaron conmigo, y a los que están tomando el relevo. Anna Lupon, mi compi FPU desde el día 0, que me ha ayudado tanto estas últimas semanas; Pol T., gracias por aguantar a mi lado hasta literalmente; Silvia, Ada, Txell, y Núria de C., mil gracias por vuestra ayuda, porque sois a las que más he hecho sudar científicamente; Daniel, Myrto, y Astrid, gracias por aparecer en el momento oportuno; Eneko y Pau G., a pesar de la maldición FPU, hay luz al final del túnel; Núria Catalán, Aurora, Lluís, y Max, gracias también a vosotros por aguantarme; mis dos satélites favoritos, Laura y Txell B.; y a todos los demás: Alba, Pol C., Dani, Isis, Esther, Bet, Claudia, Safont, Mari, Julio, Eusebi, Jaime,… esta tesis también es vuestra.

Quería agradecer a Arsenio Villarejo que me introdujera en la investigación y me enseñara lo que es hacer ciencia. Por todas las veces que te he recordado, y porque hubiera sido un placer volver a trabajar contigo.

Ahora también recuerdo que decidí realizar la tesis doctoral en una universidad, y no en un centro de investigación, por el amor a la docencia. Mil gracias a los excelentes profesores universitarios que he tenido el placer de conocer, que aunque pocos, me han marcado. Gracias a ti Núria Bonada por cederme tus horas de *Gestión integrada de cuencas* aquel curso y permitirme así experimentar la docencia en una materia en la que tenía algo que aportar. Gracias también a Eusebi y Joan Gomà por permitirme aprender de vosotros.

Gracias a todos los que me han ayudado desde fuera del departamento, desde más allá de la ciencia. A los matercianox!!!, por ser teenagers: Lucía, Andreita, JD, Patri, Peitrix, Erikota, Anox, Luis, Jevi, y Ana GoGo. A los UAMbientólogos, por seguir mi tesis desde la distancia: Vir, Ainhoa, María, David, Diego, Marta, Ana, y Gloria. Gracias a María G., por hacer su tesis cerquita mío. A todos los habitantes del hostal Olzinelles: Lucía, Pecu, Max, Mireia, Erika, Sasha... A Reflectantes, por activarme. A mis amigas de mi pueblo, que aunque la mayoría aún no saben muy bien que he estado haciendo, siempre se han preocupado por "el pez" y por "las cajas". Gracias por ser incondicionales. Y gracias a Toni, jardinero de rosas, domesticador del zorrito, al que agradezco mucho más de lo que admito. GRACIAS.

Gracias a toda mi familia. En especial a mis padres, por dejarme hacer siempre lo que he querido (como esta tesis) y por trasmitirme la inquietud por el saber, a mis hermanos, sobre todo al artista que se ha currado cinco cuadros para que los pusiera en esta tesis, y a los nuevos principitos: Diego, Mateo y el que está a punto de llegar.

Gracias a todo el mundo que en su día a día cuida la naturaleza, y todo aquel que se dedica a conocerla, conservarla, o protegerla. Gracias a los ecólogos que también son ecologistas.

Y gracias a ti, por leerme.

Barcelona, 4 de diciembre de 2015

He tenido una beca-contrato de Formación de Profesorado Universitario (FPU) del Ministerio de Educación, Cultura y Deporte (AP-2009-1470) y actualmente la prestación por desempleo. Parte de la investigación llevada a cabo ha sido financiada por el proyecto FURIMED-2 (CGL2008-03388/BO). Además, he tenido dos ayudas asociadas a la beca FPU para realizar estancias breves y tres becas de movilidad de la Universidad de Barcelona para asistir a congresos.

Advisors’ report

Dr. Narcís Prat Fornells and Dr. Maria Rieradevall Sant, advisers of the PhD thesis entitled “Fire legacies in Mediterranean streams: the key roles of the riparian canopy and the top predator on food webs", certify that the dissertation presented here has been carried out by Pablo Rodríguez Lozano. The PhD candidate is the main author of the 4 chapters, and has acted as the principal researcher in all tasks regarding them (conceiving the research objectives, designing the studies, executing the field experiments, performing the lab analyses, analyzing the data, interpreting the results, writing the manuscripts, and reviewing and editing during the publication process).

We also guarantee that none of the information contained here will be used to elaborate other PhD thesis. Below, we detail the publication status of the chapters and indicate the impact factor (ISI Journal Citation Reports® Ranking) of the journals where the chapters have been published or submitted.

Chapter 1 (published)
Long-term consequences of a wildfire for leaf-litter breakdown in a Mediterranean stream.
Pablo Rodríguez-Lozano, Maria Rieradevall, Marius Andrei Rau, & Narcís Prat
Freshwater Science (2015), vol. 34(4): 1482–1493.
This paper is part of a *Special Issue* about *Fire effects on aquatic ecosystems*.
Impact factor (2014): 1.941; Q2 Ecology (68/145); Q2 Marine & Freshwater Biology (33/105)

Chapter 2 (published)
Small but powerful: top predator local extinction affects ecosystem structure and function in an intermittent stream.
Pablo Rodríguez-Lozano, Iraima Verkaik, Maria Rieradevall, & Narcís Prat
PLoS ONE(2015), vol. 10(2): e0117630
Impact factor (2014): 3.234; Q1 Multidisciplinary Sciences (9/57)

Chapter 3 (under revision)
A trait-based approach reveals the feeding selectivity of a small endangered Mediterranean fish.
Pablo Rodríguez-Lozano, Iraima Verkaik, Alberto Maceda-Veiga, Mario Monroy, Adolfo de Sostoa, Maria Rieradevall, & Narcís Prat
Submitted to *Ecology and Evolution*.
Impact factor (2014): 2.320; Q2 Ecology (63/145)

Chapter 4 (to be submitted)
Top predator absence enhances leaf-litter breakdown in a Mediterranean stream.
Pablo Rodríguez-Lozano, Maria Rieradevall, & Narcís Prat
To be submitted to *Functional Ecology*
Impact Factor (2014): 4.828; Q1 Ecology (15/145)

For all of the above, we consider that the work of the PhD candidate grants him the right to defend his PhD thesis in front of a scientific committee.

Barcelona, December 2015

In memoriam

Dr. Narcís Prat Fornells
Catedrático de Ecología
Universidad de Barcelona

Dra. Maria Rieradevall Sant
Profesora Titular
Universidad de Barcelona

Contents

List of figures

List of tables

Summary

Wildfires are expected to increase in frequency and intensity because of climate change and changes in land use and management. In last decades, the research on fire effects on aquatic systems has grown, but it has been mainly conducted in the short- and mid-term (<5 years). Therefore, longer time frames are needed to assess fire effects on freshwater ecosystems. For instance, fire impacts on riparian and upland forests can be long-lived and wildfires often extirpate fish populations from streams, which h may not recover due to barriers within the river network. Regarding fish extirpations, it is worth noting that freshwater fish are one of the most threatened fauna worldwide, especially the small-bodied species. In this context, the overarching goal of this PhD thesis was to investigate the long-term effects of a wildfire in Mediterranean streams. Specifically, this PhD thesis covered the indirect effects of two fire legacies: (1) the riparian canopy reduction and (2) the local extinction of the top predator in these streams, the endangered small-bodied fish *Barbus meridionalis*.

The findings of this PhD thesis showed how the fire legacy in the riparian forest accelerated leaf-litter breakdown in an intermittent Mediterranean stream eight years after fire. The opening of the riparian forest canopy by fire increased light levels and water temperatures and reduced terrestrial-to-aquatic litter inputs. The increased water temperatures engendered by removal of canopy cover enhanced microbial mediated leaf breakdown. The reduction in leaf-litter inputs probably led to lower benthic organic matter levels, bringing to the observed increased shredder aggregation in leaf packs, thereby accelerating leaf breakdown rates.

Our results demonstrated that the apex consumer was functionally irreplaceable, its local extinction led to the loss of an important functional role that resulted in major changes to the ecosystem's structure and function. Our mesocom experiment showed that *Barbus meridionalis* absence led to ‘mesopredator release’, and also to ‘prey release’ despite intraguild predation, which contrasted with traditional food web theory. Top predator extirpation also changed whole macroinvertebrate community composition and increased total macroinvertebrate density. Regarding ecosystem function, periphyton primary production decreased in apex consumer absence. Moreover, we studied the feeding ecology of *B. meridionalis* from a functional perspective. Our results indicated that prey morphological and behavioral traits may explain prey vulnerability to predation. Specifically, the trait-based analysis showed that 10 of the 13 traits tested significantly influenced food choice (e.g. body size, concealment, locomotion, aggregation tendency, feeding habits). In addition, the leaf bags experiment confirmed that top predator absence enhanced leaf-litter breakdown, which was caused by the increase in shredder and scraper biomass in the absence of the predatory fish top-down control. Fish absence reduced leaf fungal biomass, but did not decrease microbially mediated leaf breakdown. These results suggested that leaf fungal biomass was stimulated from the bottom-up through nutrient recycling by the top predator.

This PhD thesis demonstrated that past fires may have current influence on the structure and function of Mediterranean streams. Moreover, our findings evidenced that intermittent streams can be affected by the consequences of apex consumers’ extinctions, and that the loss of small-bodied top predators can lead to complex ecosystem changes. This PhD thesis interconnected several current topics in ecology research (i.e. fire effects on aquatic systems, top predator declines, and intermittent streams). Its relevance lies in the projected increase in fires in the Mediterranean region and in the current high extinction risk of small-bodied fish in freshwater ecosystems.

Resumen

Debido al cambio climático y a los cambios en el uso y la gestión de la tierra, se espera que la frecuencia e intensidad de los incendios forestales aumente. En las últimas décadas, la investigación sobre los efectos de los incendios sobre los ecosistemas acuáticos continentales ha ido incrementando, pero la mayoría solo cubre las consecuencias del fuego a corto y medio plazo (<5 años), por lo que se necesitan estudios con series temporales más largas. Por ejemplo, la vegetación de la cuenca fluvial y de la zona de ribera pueden tardar décadas en recuperarse tras un incendio y los incendios suelen extirpar las poblaciones de peces en los ríos que afectan, los cuales pueden tener dificultades para la recolonización por la existencia de barreras. En este sentido, cabe la pena destacar que los peces de agua dulce son uno de los grupos faunísticos más amenazados en todo el mundo, especialmente las especies de pequeño tamaño. En este contexto, el objetivo general de esta tesis doctoral fue investigar los efectos a largo plazo de un incendio forestal en ríos mediterráneos. En concreto, esta tesis doctoral se centra en los efectos de dos legados del fuego: (1) la reducción de la cobertura del bosque de ribera y (2) la extinción local del superdepredador en estos ríos, *Barbus meridionalis*, un pez amenazado de pequeño tamaño.

Los resultados de esta tesis doctoral probaron cómo el legado del fuego en el bosque de ribera aceleró la descomposición de la hojarasca en un arroyo mediterráneo intermitente ocho años después del incendio. La disminución de la cobertura riparia por el fuego aumentó la disponibilidad de luz y la temperatura del agua y redujo la entrada de hojas desde el ecosistema terrestre. Este incremento en la temperatura del agua aceleró la descomposición de hojas mediada por microorganismos. La reducción de los subsidios terrestres probablemente condujo a una menor disponibilidad de materia orgánica en el bentos fluvial, produciendo el efecto observado de agregación de los macroinvertebrados trituradores en la hojarasca, lo cual aceleró la tasa de descomposición de las hojas.

Nuestros resultados demostraron que el superdepredador era funcionalmente insustituible, su extinción local conllevó la pérdida de su papel funcional dando lugar a cambios complejos en la estructura y función del ecosistema. Nuestro experimento de mesocosmos mostró que la ausencia de *Barbus meridionalis* provocó el incremento de los depredadores, y también de los consumidores primarios, lo que contrastó con la teoría tradicional sobre ecología de redes tróficas. La extinción local del depredador también cambió la composición de la comunidad de macroinvertebrados y condujo a una mayor densidad total de macroinvertebrados. En cuanto al funcionamiento del ecosistema, la producción primaria del perifiton disminuyó en ausencia del superdepredador. El estudio de la ecología trófica de *B. meridionalis* desde una perspectiva funcional reveló que los rasgos morfológicos y de comportamiento de los macroinvertebrados pueden explicar su vulnerabilidad a la depredación. En concreto, el análisis basado en el rasgos funcionales de los macroinvertebrados indicó que la depredación por *B. meridionalis* dependió de al menos 10 de los 13 rasgos testados (p.ej. tamaño corporal, locomoción, tendencia a la agregación, hábitos de alimentación). Esta tesis también confirmó que la ausencia del superdepredador modificó otra función clave del ecosistema, aceleró la descomposición de las hojas debido al aumento de macroinvertebrados trituradores y raspadores en ausencia del control de depredación de *B. meridionalis*. La ausencia del superdepredador resultó en una mayor biomasa fúngica en las hojas, lo que sugirió que la biomasa fúngica fue estimulada a través del reciclaje de nutrientes por el superdepredador.

Esta tesis doctoral ha demostrado que los incendios del pasado pueden influir en la estructura y función actual de los ríos mediterráneos. Además, también ha evidenciado que los ríos intermitentes pueden verse afectados por las consecuencias de la extinción de sus superdepredadores, ya que la extinción local de un superdepredador de pequeño tamaño puede provocar cambios complejos en los ecosistemas. Esta tesis doctoral interconectó varios temas contemporáneos del campo de la ecología (los efectos de los incendios en los ecosistemas acuáticos, la extinción de superdepredadores, y los ríos intermitentes). Su relevancia radica en el incremento esperado de los incendios forestales en la región mediterránea y en el alto riesgo de extinción de los peces de pequeño tamaño en los ecosistemas acuáticos continentales.

General introduction

The river and its valley

Holism, which comes from the Greek *holos* meaning whole, all, total, is a philosophical concept that can be sum up by the Aristotle's phrase: "the totality is not, as it were, a mere heap, but the whole is something besides the parts…" (*Metaphysics*, Book H, 1045: 8–10). Contrarily to reductionism, holism postulates that the constitutive characteristics of a *system* are not explainable by the characteristics of its isolated parts. Therefore, the characteristics of the *system*, compared to those of its parts, appear as new or emergent. This conception also highlights the interconnectivity and interdependency among parts. The field of ecology needs for a holistic approach. Hence, a *system* is only understandable if we contextualize it within its phenomenological integrity (Bergandi & Blandin 1998).

The holistic approach was not applied to river science since the 70s. Hynes (1975) recognized that ecologists started to study streams not as separate aquatic phenomena, but as parts of the valleys they drain, concluding that "in every respect, the valley rules the stream". For instance, he explained that many headwaters streams are heterotrophic and derive most of their energy from upland via terrestrial subsidies, which was an important scientific advance in the study of stream ecology and will be one of the main aspects analyzed along this PhD thesis. Since then, scientists have adopted a catchment integrative view of stream ecology, based on the idea that a catchment is a topographically and hydrologically defined unit (Allan, Erickson & Fay 1997). The establishment of landscape ecology discipline in early 80s also influenced in the consideration of streams and rivers as part of wider landscapes. Special importance has been given to the ecological consequences of rivers location and how river attributes depend on the characteristics of the surrounding landscape mosaic and their interactions (Fausch *et al.* 2002; Wiens 2002).

Nowadays, we know that the geology and topography of the valley, the soil porosity, and the riparian coverage are important factors for determining the hydrological regime of a river, its physicochemical characteristics, and light levels. Hence, changes in land use due to human activities can influence aquatic ecosystems and affect stream ecology in several aspects: hydrology, geomorphology, temperature and light regimes, water chemistry (i.e. nutrients, pollutants, salinity), organic matter availability, ecosystem processes, and biological communities (Allan 2004; Burcher, Valett & Benfield 2007; Cooper *et al.* 2013). Similarly, fires deeply modify the valleys within streams and rivers flow, thus we may expect that the changes caused by fire in catchments should be translated to the running waters therein.

Fire is recognized as one of the main factors that shape ecosystems (Cowling *et al.* 2005; Bond & Keeley 2005), and as key factor to understand many of the world's biomes distribution (Bond & Keeley 2005). Wildfires are natural disturbances that are expected to increase in frequency and intensity because of climate change and changes in land use and management (Bowman *et al.* 2011; Dury *et al.* 2011; IPCC 2014). In the Mediterranean region, recent modelling work (Dury *et al.* 2011) projected that the area burned by wildfires will increase 3-5 times at the end of this century compared to present. Although fire effects on terrestrial ecosystems are well-studied, our knowledge about fire effects on aquatic systems is still limited. Research on this topic has been primarily conducted in Western US, while impacts on freshwater ecosystems from Mediterranean region are scarce (Verkaik *et al.*

2013a). Therefore, a better understanding of fire impacts on aquatic ecosystems is urgently needed.

Fire impacts on streams

Fire may act as prime driver of change in the physical, chemical, and biological characteristics of streams, its cascading effects on these ecosystems being highly complex (see Fig. I.1). Consequently, the effects of fire on aquatic ecosystems are usually separated into direct and indirect effects (Minshall 2003). Direct impacts are often reduced to ash deposition, temperature increase (direct natural effects), and the introduction of contaminants such as fire retardants (direct human-induced effects). The impacts of ash and pollutants depend on water flow, being diluted if they are transported downstream, but deposited in pools if surface flow is low or nonexistent. Fire may burn riparian areas, opening canopy and, indirectly, increasing light levels and water temperature (Gresswell 1999; Koetsier, Krause & Tuckett 2010). These changes may mediate fire effects on streams, due to the strong connectivity between streams and their riparian areas.

Rainstorms after fire usually have major impacts on freshwater ecosystems because sediments, nutrients, and organic matter are washed from the catchment and quickly transported to the stream channel (Wondzell & King 2003; Pausas *et al.* 2008; Coombs & Melack 2013). Consequently, fire may change completely water chemistry by decreasing oxygen levels and by increasing the concentration of suspended solids, phosphate, ammonium, nitrate, total and dissolved organic carbon, and other parameters (Earl & Blinn 2003; Vila-Escalé 2009; Rhoades, Entwistle & Butler 2011; Coombs & Melack 2013). This decrease in water quality not only alters aquatic communities and ecosystem processes, but also can affect the water supply, being of particular concern to water resource managers. Fire also alters hydrology by reducing infiltration, evapotranspiration, and interception through its impact in soil porosity and vegetation cover (Inbar, Tamir & Wittenberg 1998; DeBano 2000; Coombs & Melack 2013). Mid- and long-term studies, despite being scarce, have showed high variability in the recovery patterns of the water physicochemical parameters. It has been shown that discharge, sediment levels, and water chemistry in streams draining burned catchments may recover during the first postfire year, or remain altered during >5 years (Earl & Blinn 2003; Cerdà & Doerr 2005; Meixner *et al.* 2006; Mayor *et al.* 2007; Rhoades *et al.* 2011).

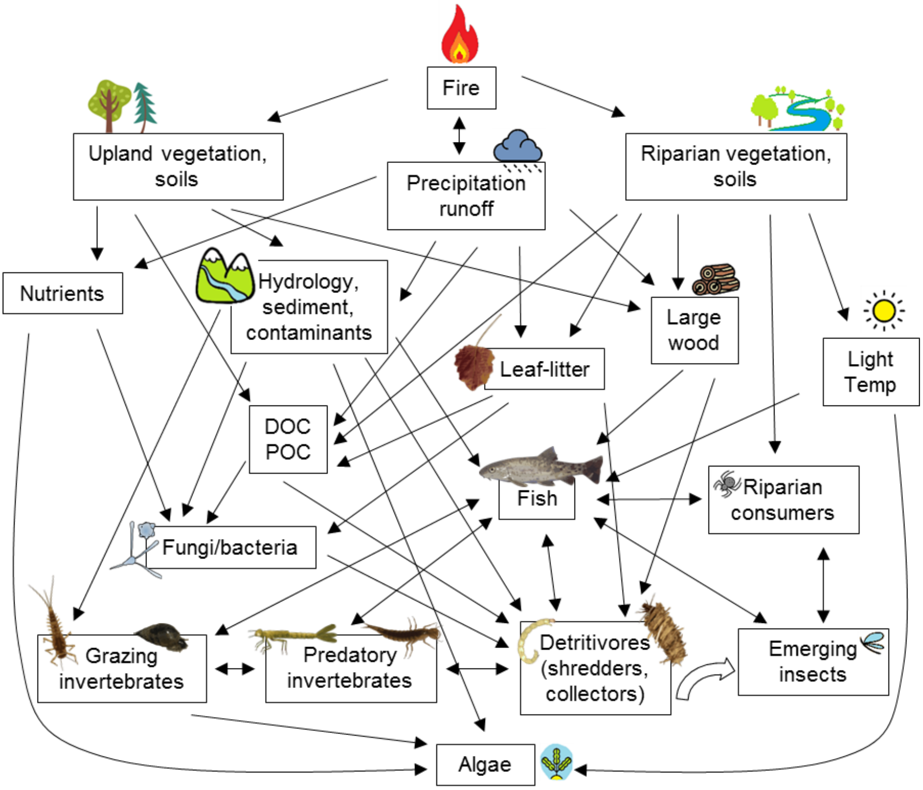


Figure I.1 Path diagram showing probable cause–effect relationships leading from fire to stream communities. Temp = temperature, DOC = dissolved organic carbon, POC = particulate organic carbon. Adapted from Bixby *et al.* (2015).

Aquatic biological communities may be dramatically affected by scouring floods after fire. Fire may reduce (Sestrich, McMahon & Young 2011) or completely extirpate (Rinne 1996; Gresswell 1999; de Sostoa *et al.* 2006) fish populations, but recolonization may occur if streams do not have barriers to fish access (Gresswell 1999). Macroinvertebrate density, richness, and diversity usually decrease after fire, but other studies have shown no changes or even an increase in these parameters (Roby & Azuma 1995;

Minshall, Robinson & Lawrence 1997; Mellon, Wipfli & Li 2008; Hall & Lombardozzi 2008; Vila-Escalé 2009; Verkaik *et al.* 2013b). This variability in the macroinvertebrate community responses can be attributed to fire severity as well as to the magnitude, frequency, and timing of following storms (Verkaik *et al.* 2013a). Macroinvertebrate communities after fire often shift to species with high aerial dispersal rates, small size, fast development, and generalist food habits (i.e. *r*-strategists species) (Minshall *et al.* 1997; Minshall, Royer & Robinson 2001; Vieira *et al.* 2004; Verkaik *et al.* 2013b). Wildfire effects on algal and amphibians communities are less explored (but see Pilliod *et al.* 2003; Farrés-Corell 2005). These biological communities (i.e. algae, macroinvertebrates, and amphibians) can have a fast recovery after fire in Mediterranean streams compared to other climatic regions (1–4 years in Mediterranean streams vs 5–10 years in non-Mediterranean streams) because most species in Mediterranean streams are disturbance-adapted due to seasonal and interannual hydrological variability (Verkaik *et al.* 2013a).

Regarding the organic matter subsidies, terrestrial inputs of leaf-litter to streams appear to increase immediately after fire and to decrease over the following years (Britton 1990; Jackson, Sullivan & Malison 2012). Burned leaves may decompose at similar (Gama *et al.* 2007) or faster (Vieira, Barnes & Mitchell 2011) rates than unburned leaves. Fire also may alter leaf breakdown rates in the long-term (Koetsier *et al.* 2010). Although leaf-litter inputs fuel many stream food webs, the fire effects on organic matter inputs and processing still remains a challenge for stream ecologists. In general, most of the studies analyzing the effects of fire on freshwater ecosystems have focused on state variables, overlooking rate variables such as leaf breakdown or stream metabolism (but see Robinson, Uehlinger & Minshall 2005; Betts & Jones Jr. 2009). Consequently, a recent review (Verkaik *et al.* 2013a) recommended conducting additional studies on fire effects on leaf breakdown in aquatic ecosystems.

Most research on fire effects on streams have mainly been conducted in the short- and mid-term (<5 years). However, it has been demonstrated that fire can have legacy effects on streams (but see Robinson *et al.* 2005; Koetsier *et al.* 2010). Legacy effects can be defined as the consequences of disturbances that continue to influence environmental conditions long after the appearance of the disturbance. Therefore, longer time frames are needed to evaluate the effects of fire in freshwater ecosystems.

Fire impacts on streams: *Sant Llorenç del Munt* study case

On August 2003, a forest fire affected the Sant Llorenç del Munt i l'Obac Natural Park, burning 4543 ha. The fire had medium intensity and was the consequence of the dry conditions over the previous months (temperatures of 39ºC, 7% of relative humidity, and the accumulated precipitation in spring was 8 mm) combined with specific wind regimes. The burned area had high conservation and recreational interest; riverside, Holm oak and pine woods were affected (Guinart 2007). The Freshwater Ecology and Management (F.E.M.) research group has studied the impacts of this wildfire in streams, being pioneer in this topic in Europe.

Sant Llorenç del Munt i l'Obac Natural Park is a protected area declared in 1987 and it is situated in Barcelona province (50 km inland from Barcelona, NE Spain), currently occupying 13694 ha. Vegetation is dominated by Holm oak (*Quercus ilex* L.) and Aleppo pine (*Pinus halepensis* Miller) forests and Mediterranean shrubs. The Natural Park also has some minor areas of cereal cultivation. The region has a Mediterranean climate with mild winter and warm spring and summer. Rainfall is irregular and intense, and it mostly occurs in winter, while summer is normally very dry. This hilly area is characterized by a calcareous geology, with alternating highly permeable and less permeable substrates where springs are located. As a consequence of this two characteristics (i.e. climate and geology) most streams in the area are reduced to isolated pools or even dry completely during the dry season (usually summer), that is, most stream network is intermittent (Bonada, Rieradevall & Prat 2007b).

The study of fire effects on the streams of Sant Llorenç del Munt i l'Obac Natural Park began in 1993, when the late Prof. Maria Rieradevall took a series of water and biological samples within the area short after a wildfire event. This study was preceded by other describing the communities present in the Natural Park in 1987 (Real, Rieradevall & Prat 1989). Some of the sampled sites were previously part of the biomonitoring works that began in 1979 leaded by Prof. Narcís Prat (Prat *et al.* 1982; Prat, Puig & González 1983). Although the 1993 study of the fire effects on intermittent streams is not published, many ideas were gathered and have been used by the F.E.M. research group after the 2003 fire. Just few days after the 2003 fire, the first water and macroinvertebrate samples were taken, and since then, Gallifa and Vall d'Horta streams have been studied as fire-affected streams.

The 2003 fire and the following rains decreased water quality, homogenized aquatic habitats, and extirpated stream macroinvertebrate communities (Vila-Escalé 2009). The first rains transported large quantities of ash and mud from burned watersheds into and along the streams, filling pools with mud and ash, and killing high densities of both native and introduced fish species (de Sostoa *et al.* 2006). Afterwards, heavy rains caused floods, removing most mud and ash from stream channels. The concentration of most physicochemical variables (total and dissolved organic carbon, phosphate, cations) increased after fire, but sharply decreased in 2 months. Nitrate, suspended solids, and polycyclic aromatic hydrocarbon concentrations remained high during the first year and presented sporadic peaks related to runoff and soil erosion following precipitation events (Vila-Escalé *et al.* 2007b; Vila-Escalé, Vegas-Vilarrúbia & Prat 2007a). Fire reduced diatom richness, increased disturbance tolerant species abundance, and modified community composition. Diatom richness recovered during the first year, but species composition remained different (Farrés-Corell 2005; Vila-Escalé 2009). Some amphibian species were affected, but all recolonized streams by 3 years after fire (Campeny 2007).

Macroinvertebrate communities disappeared due to the wildfire. Dipterans and coleopterans were the first colonizers. The monitoring of a headwater stream located within a burned catchment showed that family richness and macroinvertebrate abundance increased, and the macroinvertebrate communities were becoming similar during the first postfire year to those found in a control stream in terms of both composition and functional traits (Vila-Escalé *et al.* 2007c; Vila-Escalé 2009). Later on, Verkaik (2010) investigated if the responses of the macroinvertebrate communities in that single stream could be transposed to other Mediterranean streams and if were present on a more extended time frame. To achieve this aim, eight sites were sampled annually for 5 years. Neither taxa richness nor total abundance differed among fire-affected and control streams 2 months after fire. However, the wildfire changed the composition and the biological traits of macroinvertebrate communities. An increase in *r*-strategy taxa was observed during the year following fire. However, drought was a prevailing disturbance factor, i.e. macroinvertebrate assemblages responded more strongly to interannual hydrological variability than to fire (Rodríguez-Lozano 2010; Verkaik *et al.* 2013b). After 5 years, macroinvertebrate communities had recovered from fire, but a few individual taxa (Mollusca and some Odonata) were still less abundant in fire-affected than in control streams.

As previously mentioned, stream fish communities were extirpated by the wildfire in those streams draining burned catchments, potentially due to the chemical changes that occurred during the firsts rainfall events. Two years after fire, native fish species, *Barbus meridionalis* (A. Risso, 1827) and *Squalius cephalus* (L., 1758), were observed in some of the affected streams, but their densities and biomasses remained low compared to prefire values and to those in control streams (de Sostoa *et al.* 2006). Currently, several fire-affected streams that were inhabited by *B. meridionalis* before the fire still remain fishless most likely due to natural and human barriers. *Barbus meridionalis* (see Fig. I.2) is an endemic species to NE Spain and SE France, and is currently listed as 'vulnerable' in the Spanish Red Book (Doadrio 2001), 'near threatened' in the IUCN and is also included in Annexes II and V of the European Union Habitats Directive and in Appendix III ('protected fauna species') of the Bern Convention. Similar to other freshwater fish species, the list of threats include water pollution, water abstraction, dam constructions, introduced species, and alterations in the habitat (Doadrio *et al.* 2011; Maceda-Veiga 2013). Additionally to this species inherent value, the local extinction of *B. meridionalis* due to the wildfire may be important at ecosystem level as a fire legacy effect: the extirpation of this fish species may have had complex indirect effects on the biological communities and ecosystem processes in these streams.

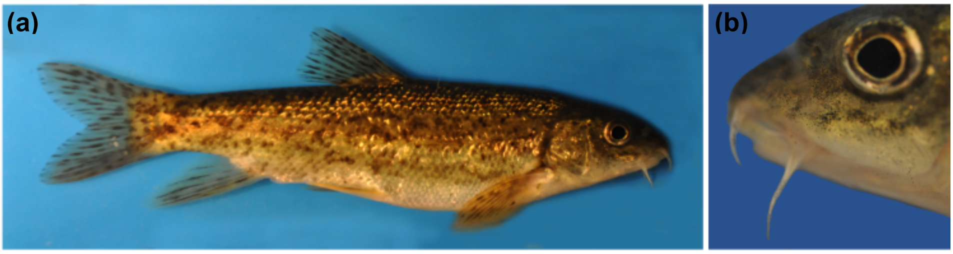


Figure I.2 Photographs of: *Barbus meridionalis* (a) and detail of the barbels (b).

Trophic downgrading of ecosystems

Our planet is experiencing a large biodiversity loss, i.e. the Sixth Mass Extinction, which is characterized by the loss of apex consumers (Purvis *et al.* 2000; Duffy 2002; Estes *et al.* 2011; Schneider & Brose 2013). Several studies have indicated that top-predator loss may trigger ecosystem changes that are complex, unpredictable, and largely unknown (Marshall & Essington 2011; Estes *et al.* 2011). Consequently, a current major challenge is to better understand the responses of ecosystems to current top predator declines (Sutherland *et al.* 2013).

Predation is an important trophic interaction that has implications for biological populations, communities, and ecosystems. The top-down force that predators exert on their prey can be propagated through the trophic web, leading to strong indirect effects (i.e. trophic cascades) (Hairston, Smith & Slobodkin 1960; Polis *et al.* 2000; Terborgh & Estes 2010). Trophic cascades have been documented in terrestrial, freshwater, and marine ecosystems, from the poles to the tropics, and most have focused on grazing systems (Terborgh & Estes 2010). Ecosystems loss of apex consumers may alter the intensity of herbivory and, consequently, the abundance and/or the composition of primary producers. Although less studied, trophic cascades have also been observed in detritus-based systems, such as soil forests and forested headwater streams (Miyashita & Niwa 2006; Greig & McIntosh 2006). Trophic cascades can be density-mediated through the reduction of prey density, but also trait-mediated when the presence of the predator modifies the prey foraging behavior (Schmitz, Krivan & Ovadia 2004;

Preisser, Bolnick & Benard 2005). Besides, the extinction of top predators is often associated with an increase in mesopredators (i.e. any mid-ranking predator in a food web), which may lead to complex indirect effects in ecosystems (Ritchie & Johnson 2009; Prugh *et al.* 2009; Brashares *et al.* 2010).

Moreover, apex consumers are involved in many non-trophic interactions (Kéfi *et al.* 2012). Top predators may play a key role in nutrient recycling through the excretion and egestion of nutrients (Vanni 2002; Schmitz, Hawlena & Trussell 2010). Apex consumers can change the flow of both organisms and organic matter in and out of their systems, having important effects across ecosystem boundaries (Knight *et al.* 2005; Kéfi *et al.* 2012). Through indirect pathways, the loss of the top predator can alter diverse ecosystem aspects, such as disease incidence (Lafferty 2004) and the chemical composition of the atmosphere and water (Schindler *et al.* 1997; Moore *et al.* 2007). Hence, the extirpation of top predators may conduct to ecological network simplifications caused by the loss of trophic and non-trophic links, leading to complex ecosystem consequences.

Most of the research regarding the consequences of the extinction of top predators have been focused on large-bodied terrestrial mammals (Prugh *et al.* 2009; Beschta & Ripple 2009; Ordiz, Bischof & Swenson 2013; Ripple *et al.* 2014) and on large marine predators (Heithaus *et al.* 2008; Ritchie & Johnson 2009), overlooking freshwater ecosystems. Moreover, most studies usually associate large-bodied species to the top of the food webs and small-bodied species to lower trophic levels (Marshall & Essington 2011; Säterberg, Sellman & Ebenman 2013), even positing that large-bodied species are at much greater extinction risk than smaller species (Cardillo 2003).

However, aquatic species (marine and freshwater) seem more vulnerable to extinction than terrestrial species, being freshwater fish considered one of the most threatened fauna worldwide (Jenkins 2003). In Europe, a recent study has predicted that fish will be heavily impacted by climate change, with more than 40% of the species under threat (Markovic *et al.* 2014). In marine ecosystems, large fish are the most endangered, but in freshwater ecosystems, small-bodied fish exhibit greater risk of extinction than their larger-bodied counterparts (Olden, Hogan & Zanden 2007). These small-bodied fish species often act as top predators in several freshwater ecosystems (i.e. intermittent rivers, headwater permanent streams, and ponds) (Meyer *et al.* 2007; Reich *et al.* 2010; Brucet *et al.* 2012). For instance, *Barbus meridionalis* act as apex consumer in several Mediterranean intermittent streams of Sant Llorenç del Munt Natural Park. Despite their high extinction risk, small-bodied freshwater fish species are often overlooked in research and conservation management even when considered threatened, probably because they usually do not have a commercial value (Williams 2006; Braga, Bornatowski & Vitule 2012; Saddlier, Koehn & Hammer 2013) and inhabit ecosystems that also are neglected by conservation and management (Boix *et al.* 2012; Nikolaidis *et al.* 2013). Consequently, it remains unknown if the loss of small-bodied freshwater fish can be equivalent to local extinctions of larger apex consumers in other ecosystems (e.g. the arctic fox, wolf, jaguar, sea otter or large reef fish (Beschta & Ripple 2009; Estes *et al.* 2011; Ripple *et al.* 2013).

Intermittent rivers

Intermittent rivers and streams are those that cease surface flow at some point in space and time along their course. This nomenclature (i.e. intermittent) includes also other terms such as seasonal, ephemeral, temporary, episodic, or semi-permanent. Intermittent rivers are a heterogeneous category that covers a wide range of hydrological regimes. For instance, some intermittent streams can be dry most of the time, presenting running water only after heavy rains; while other streams can flow during part of the year, being reduced to disconnected pools some months and never become dry (see Fig. I.3). The variability in hydrological conditions in these systems leads to a transition through different assemblages of aquatic habitats along time (i.e. aquatic states) (Gallart *et al.* 2012). Moreover, high interannual variability not just intraannual, may affect intermittent rivers.

Figure I.3 Photographs of intermittent rivers from Spain: Vall d'Horta (a,c), Monlleó (b), Córcoles (d), Daró (e), Gigüela (f), and Matarranya (g).

Intermittent rivers are present in all climate areas. These systems comprise approximately one half of the global river network and are projected to increase due to climate change and water abstraction for human use (Carlisle, Wolock & Meador 2011). However, most of the river research has been mainly focused on permanent rivers, and thus, the current paradigms in river science and management have emerged and developed in these ecosystems. The principles of biodiversity conservation, integrated water resource management, and water quality control neglect intermittent rivers (Prat *et al.* 2014). Consequently, water legislation and regulations such as the European Water Framework Directive (EU-WFD; European Communities, 2000) or the US Federal Water Pollution Control Act (i.e. the Clean Water Act) generally ignore these systems (Nikolaidis *et al.* 2013; Mazor *et al.* 2014).

The ecology of intermittent streams was early investigated by H. B. Noel Hynes through the PhD thesis of D. Dudley Williams (Williams & Hynes 1976, 1977), who more recently wrote a key book on this topic (Williams 2006). The number of studies focusing on intermittent rivers has grown exponentially during 1990-2014 (Datry, Arscott & Sabater 2011; Leigh *et al.* in press). Most research in these systems has focused on how hydrological variability (i.e. the change to one aquatic state to another) shapes biological communities (Bonada *et al.* 2006; Davey & Kelly 2007; García-Roger *et al.* 2011; Rocha, Medeiros & Andrade 2012; Datry *et al.* 2014) and biogeochemical processes (Hladyz *et al.* 2011; von Schiller *et al.* 2011; Corti *et al.* 2011; Dieter *et al.* 2011; Timoner *et al.* 2012). However, despite this increasing concern for intermittent rivers, little is known about whether these systems are threatened by some problems that affect most ecosystems worldwide, such as invasive species or top predator loss. In fact, a review published this year (Leigh *et al.* in press) highlights that one of the future research lines in intermittent rivers research must focus on how anthropogenic stressors and future changes may affect these systems.

The role of top-down structuring forces has been largely overlooked in intermittent rivers research. Intermittent rivers often lack large aquatic consumers that are often considered to be top predators, and instead, are typically inhabited by predatory invertebrates and small-bodied fish (Meyer *et al.* 2007; Reich *et al.* 2010). These systems were initially viewed as severe environments for fish species (Williams & Coad 1979) and have been considered a refuge from vertebrate predation (Williams 1996; Meyer *et al.* 2007). Some studies have even suggested that predatory invertebrates have lower abundances in intermittent than in permanent streams (Bogan, Boersma & Lytle 2013); while others indicated that predation pressure increases in these systems with stream fragmentation in isolated pools, when predatory lentic invertebrates (odonates, hemipterans, and coleopterans) replace reophilous taxa (Boulton & Lake 1992a; Stanley *et al.* 1994; Rieradevall, Bonada & Prat 1999; Bonada *et al.* 2007b).

Regarding predatory fish, several studies have shown that macroinvertebrates in intermittent rivers can be affected in terms of whole community assemblage and total density (Williams, Taylor & Warren 2003), densities of specific groups (e.g. air breathing macroinvertebrates (Closs 1996)), total biomass (Wesner 2013), and prey body condition (Love, Taylor & Warren 2005). Conversely, other studies suggest that predatory fish may not affect macroinvertebrate communities (Ludlam & Magoulick 2010). All these studies were performed in isolated pools or in pools that became isolated during the experiment, when predation pressure may reach its peak in these systems. Hence, the importance of predation in intermittent rivers during periods of flow remains unknown.

Therefore, we still ignore if intermittent rivers are affected by the current loss of top predators, and the potential ecosystem consequences derived from this worldwide problem. Given the high extinction risk of small-bodied freshwater fish that often act as top predators in intermittent streams, we hypothesized that these systems are threatened by their extinction.

Objectives

The overarching goal of this PhD thesis was to investigate the long-term effects of a wildfire in Mediterranean streams, i.e. caused by the fire legacy. In brief, this PhD thesis covered the indirect effects of two fire legacies: (1) the riparian canopy reduction and (2) the local extinction of the top predator in these streams, the endangered small-bodied fish *Barbus meridionalis*. Therefore, most part of this PhD thesis actually focused on determining if intermittent streams are threatened by the loss of top predators and on the ecosystem consequences of their extinction in terms of both structure and function. The PhD thesis has a food web approach and is mainly formed by in-stream experiments.

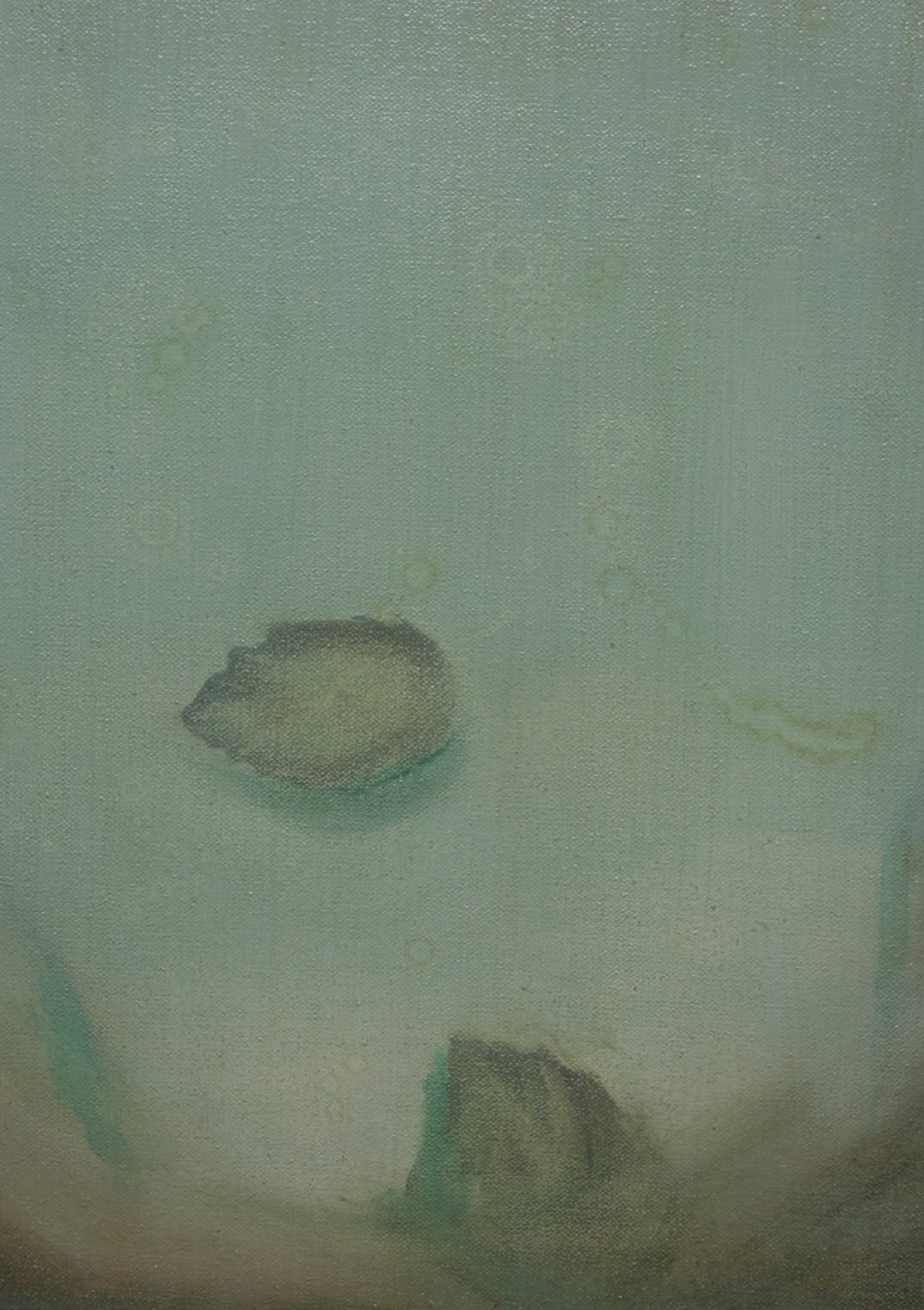
The PhD thesis is structured in four independent chapters, each one addressing a specific objective. However, the chapters are interconnected and contain cross references to other chapters. Specific objectives of each chapter are as follows.

In Chapter 1, we assessed the long-term (8 years) effects of a wildfire on leaf-litter breakdown in an intermittent stream. In particular, we differentiated between fire effects on microbial and overall leaf decomposition to better understand the mechanisms accounting for wildfire effects on this ecosystem process.

In Chapter 2, we examined the top-down effects of *B. meridionalis* by using in-stream mesocosms. Specifically, we aimed to determine whether the loss of a top predator in an intermittent stream (1) leads to a 'mesopredator release', affecting primary consumers and changing whole community structure and (2) triggers a cascade effect modifying ecosystem function (i.e. periphyton primary production).

In Chapter 3, we proposed a simple method with a functional perspective to study the feeding selectivity of predators, through the study case of *B. meridionalis.* We hypothesized that predators select their prey depending on prey morphological and behavioral traits.

In Chapter 4, we investigated the top-down effects of an endangered small-bodied fish in a detritus-based intermittent stream. In particular, we aimed to determine whether the loss of a top predator alters (1) leaf fungal biomass and leaf quality (i.e. leaf carbon:nitrogen ratio and leaf toughness), (2) macroinvertebrate assemblages colonizing leaf packs and, consequently, (3) leaf breakdown rates.



Chapter 1

Long-term consequences of a wildfire for leaf-litter breakdown in a Mediterranean stream

Wildfire frequency and intensity are expected to increase as a result of climate change, but few studies have assessed the effects of wildfires on stream ecosystem processes. The aim of our study was to examine the long-term responses of leaf-litter breakdown to wildfire in a Mediterranean stream. Riparian canopy cover was reduced by a fire, resulting in higher water temperatures and light levels and reduced leaf-litter inputs in the stream 8 years after the fire. Benthic invertebrate communities recovered quickly after the fire, and the abundances of different functional feeding groups, including shredders, were not different between streams affected and not affected by the fire after >2 postfire years. We compared microbially mediated (fine-mesh bags) and total breakdown rates (coarse-mesh bags) of *Populus alba* leaf-litter incubated in the stream affected by the wildfire and a neighboring control stream. Microbial and total leaf-litter breakdown were faster in the stream affected by the fire. Faster microbially mediated litter decomposition in the fire-affected stream could be attributed almost entirely to increased water temperatures, but total litter breakdown rates were enhanced by higher shredder abundance in coarse-mesh leaf bags in the fire-affected than in the control stream. Lower leaf-litter inputs in the fire-affected than in the control stream probably led to lower benthic organic matter levels, bringing to increased shredder aggregation in leaf packs, thereby accelerating leaf breakdown rates. Our study indicates that past wildfires can modify key ecosystem processes, such as leaf decomposition, in the long-term in Mediterranean streams.

Introduction

Wildfires are expected to increase in frequency and intensity because of climate change and changes in land use and management (Bowman *et al.* 2011; Dury *et al.* 2011; IPCC 2014). Fire is recognized as one of the main factors that shape ecosystems (Cowling *et al.* 2005; Bond & Keeley 2005), but few investigators have studied its effects on stream ecosystem processes (but see Robinson *et al.* 2005, Betts and Jones 2009, Koetsier *et al.* 2010). Leaf-litter inputs and decomposition fuel many stream food webs, and the authors of a recent review recommended conducting additional studies on fire effects on the key ecosystem process of leaf-litter breakdown (Verkaik *et al.* 2013a). Experimental studies suggest that decomposition rates of burned leaves do not differ from (Gama *et al.* 2007), or are faster than (Vieira *et al.* 2011), those of unburned leaves. Koetsier *et al.* (2010), authors of the only study we know of fire effects on stream leaf-litter breakdown in the long-term, found that litter decomposition rates were faster in fire-affected than unaffected reference streams in a temperate forest. These results evidence the need for additional studies on fire effects on stream leaf-litter breakdown in other biomes, such as Mediterranean regions, where fire is common.

Wildfires could stimulate leaf decomposition mediated by microbes by reducing riparian canopy cover, thereby increasing water temperatures (Koetsier *et al.* 2010; Boyero *et al.* 2011; Martínez *et al.* 2014; Cooper *et al.* 2015). Reductions in canopy cover also increase light levels, potentially enhancing microbial decomposition via increases in algal production on leaf-litter (Danger *et al.* 2013). Moreover, algal development on leaves could increase litter palatability, accelerating litter breakdown mediated by macroinvertebrate activity (Lagrue *et al.* 2011). In contrast, fire-induced reductions in canopy cover could increase ultraviolet radiation levels, thereby reducing leaf-litter mass loss (Pancotto *et al.* 2003; Dieter *et al.* 2011). Wildfires also could modify microbially mediated litter decomposition rates by increasing dissolved nutrient concentrations, which would accelerate decomposition (Woodward *et al.* 2012), and by increasing the scour and deposition of fine sediment, which could reduce (Benfield *et al.* 2001; Lecerf & Richardson 2010) or increase (Piggott *et al.* 2012) decomposition. However, these effects of fire on nutrients and sediment are often short-lived, so litter decomposition may not be affected over the long-term by these agents (Benfield *et al.* 2001; Earl & Blinn 2003; Vila-Escalé 2009; Lecerf & Richardson 2010; Verkaik *et al.* 2013a).

Macroinvertebrate mediated leaf breakdown could decrease after fire if shredding invertebrate density decreases (Molles 1982; Vieira *et al.* 2004; Oliver *et al.* 2012; Verkaik *et al.* 2013a; Cooper *et al.* 2015). Fire-mediated decreases in shredder density can be attributed to decreased leaf-litter inputs and retention after fire and to the vulnerability of shredders to scouring floods induced by fire (Verkaik *et al.* 2013a; Cooper *et al.* 2015). Observational (Boulton & Lake 1992b; Friberg 1997; González & Graça 2005) and experimental (Richardson 1991; Dobson & Hildrew 1992; Wallace *et al.* 1997) studies indicate that shredder densities in streams are related to organic matter availability. Knowledge about the effects of wildfires on organic matter inputs to streams is limited, but terrestrial subsidies of leaf-litter to streams appear to increase immediately after fire and decrease over the following years (Britton 1990; Jackson *et al.* 2012). On the other hand, increased stream temperatures after fire could increase shredder activity. Wildfires may have little effect on leaf breakdown mediated by macroinvertebrate activity in the long-term because the abundance and taxonomic and functional composition of macroinvertebrate communities in Mediterranean streams often recover quickly after wildfires (Vila-Escalé 2009; Verkaik *et al.* 2013a; b).

The aim of our study was to assess the long-term (8 years) effects of a wildfire on leaf-litter breakdown in a Mediterranean stream. We differentiated between fire effects on microbial and overall leaf decomposition to better understand the mechanisms accounting for wildfire effects on this ecosystem process. We conducted a decomposition experiment using *Populus alba* leaves enclosed in coarse- or fine-mesh bags incubated in a stream affected by fire and in a neighboring control stream. To assess some of the key factors potentially mediating the effects of the wildfire on stream litter breakdown, we monitored the riparian canopy cover after fire, temperature and light levels during the decomposition experiment, litterfall input over 1 year encompassing the decomposition experiment, and the abundances of macroinvertebrate functional feeding groups (FFGs) in streams draining burned and unburned catchments over the first 5 years after fire as well as in leaf bags during the experiment (8 years postfire). We hypothesized that 8 years after a wildfire: (1) microbial leaf decomposition would be faster in the fire-affected than in the control stream because of increases in water temperature and (2) macroinvertebrate mediated leaf-litter breakdown would be similar between the fire-affected and the control streams because of the rapid recovery of macroinvertebrate communities, including shredder densities, after fire.

Materials and methods

Study area

We worked in 2 adjacent first order streams, separated by <5 km. The fire-affected stream was Vall d'Horta stream (lat 41°39′15″N, long 2°4′13″E), and the control stream was Castelló stream (lat 41°40′42″N, long 2°1′49″E). The streams are in the protected area of Sant Llorenç del Munt i l'Obac Natural Park (50 km inland from Barcelona, NE Spain). This area is characterized by a calcareous geology and a Mediterranean climate, with mild winter and warm spring and summer. Rainfall is irregular and intense, occurring primarily in winter but also in spring and autumn, and summer is normally very dry. The protected area is dominated by Holm oak (*Quercus ilex* L.) and Aleppo pine (*Pinus halepensis* Miller) forests and Mediterranean shrubs (for a detailed description see Bonada *et al.* 2007b, Verkaik *et al.* 2013b). In August 2003, a wildfire burned 4543 ha of forest in the Natural Park and surrounding areas.

Both streams are tributaries of the Ripoll River in the Besòs basin, and their catchments are mainly forested, dominated by Aleppo pine with small areas of cereal cultivation (<15%). The selected 100-m reach in each stream was <2 m wide and <50 cm deep, with a bedrock stream bed. In the 2003 fire, 62.1% of the catchment of the fire-affected stream, including its riparian zone, was burned. Riparian and upland forests have not recovered completely at this site. The control stream was unaffected by the wildfire and continues to be shaded by a well-developed canopy (Table 1.1). In summer 2012, the riparian vegetation at the fire-affected site was constituted by 25 to 50% trees, 25% shrubs, and 25 to 50% annual herbs, whereas the control reach was lined by a well-developed riparian forest (>75% trees) with understory shrubs. These 2 reaches, 1 additional control reach, and 5 additional fire-affected reaches were monitored by Verkaik *et al.* (2013b) for 5 years after the fire, and we reanalyzed data from that study to estimate the responses of invertebrate functional feeding groups to fire.

Canopy cover and physicochemical measurements

Riparian canopy cover was estimated annually as part of a riparian forest quality index (QBR index; Munné *et al.* 2003) at both the control and the fire-affected sites from 2 months after the fire until summer 2012. We reanalyzed the canopy cover data collected by Verkaik *et al.* (2013b) for 2 control and 6 fire-affected reaches over the first 5 years after fire. We also made physicochemical measurements throughout the litter decomposition experiment (n = 6 times). We measured dissolved O_2, pH, and conductivity in situ with a Multiline P4 WTW meter (YSI, Yellow Springs, Ohio, U.S.A.), and we estimated discharge from mean depth, transect width, and water velocity with a flow meter (miniAir, Schiltknecht, Gossau, Switzerland). We collected water samples by hand, filtered them through GF/F Whatman filters, and analyzed them for soluble nutrients. We measured ammonium (NH_4^+-N) and soluble reactive phosphorous (PO_4^{3-}-P) with spectrophotometric methods (Murphy & Riley 1962) and nitrate (NO_3^--N) with ionic chromatography methods (EPA method 9056, USEPA 1993; UV/V KONTRON model 332, Kontron AG, Zürich, Switzerland). We deployed submersible temperature/light data loggers (HOBO Pendant, Onset Computer Corporation, Bourne, Massachusetts) under stream water to record water temperature and light intensity every 10 minutes at each site throughout the leaf bags experiment.

Benthic macroinvertebrate communities

We compared the abundances of benthic macroinvertebrate functional feeding groups at 2 control and 6 fire-affected sites over the first 5 years after the fire by reanalyzing the data collected by Verkaik *et al.* (2013b). At each site and time, macroinvertebrate kick samples were collected with a 250-µm net from all extant habitats over a standard 4-minutes period. Macroinvertebrate samples were processed as outlined below for macroinvertebrates collected from leaf bags.

Litterfall input

We measured vertical litterfall inputs with 0.28-m^2 traps (3 at the fire-affected site and 3 at the control site) placed in the riparian zone (Pozo, González & Díez 1997). Traps were made of rigid baskets 60-cm in depth and lined with a 1-mm mesh, that retained all coarse particulate organic matter (CPOM) but allowed water to escape during rain events. Litter in traps was removed 12 times during a year (4 October 2011 – 4 October 2012), with increased sampling frequency in autumn. We oven-dried litter (60°C, 72 h), and sorted it as leaves, bark and twigs, or fruits and flowers. We weighed and combusted (500°C, 12 h) this material to measure ash-free dry mass (AFDM).

Leaf-litter decomposition experiment

We conducted the leaf bag experiment in autumn–winter (23 November 2011 – 15 March 2012). We collected leaves of white poplar (*Populus alba* L.) in October 2011 just after abscission, and air-dried them at room temperature to constant mass. Leaf bags (15 × 20 cm) containing 3 g of leaves (SE = 0.06 g) were made of 2 mesh sizes: coarse (10 mm) and fine (250 µm). Coarse-mesh bags allowed macroinvertebrate colonization, thus more closely simulated natural leaf-litter breakdown processes, whereas fine-mesh bags excluded virtually all macroinvertebrates, thereby allowing us to assess the relative contribution of microbial (fungi and bacteria) activity to litter decomposition (Young *et al.* 2008).

We deployed 30 bags of each mesh type at both the fire-affected and the control sites in pools because leaves accumulated in the pools of these streams. We placed 12 additional bags in the control site for 24 h to correct for initial leaf mass losses resulting from leaching and accidental transport losses (Gessner, Chauvet & Dobson 1999). We removed 6 litter bags of each type (coarse- and fine-mesh) from each site 5, 12, 26, 58 and 113 days after deployment. At retrieval, we placed litter bags individually in Zip-lock® bags and transported them in refrigerated containers to the laboratory, where we processed them immediately. We washed the material in each litter bag, collected invertebrates on a 250-µm sieve, and preserved them in 70% ethanol. We oven-dried (60°C, 72 h) and combusted litter material (500°C, 12 h) to measure AFDM remaining.

We counted macroinvertebrates, identified them to the lowest possible taxonomic level (usually genus), and measured them using ImageJ software (version 1.47, National Institutes of Health, Bethesda, Maryland, U.S.A.). We classified taxa to functional feeding groups following Tachet *et al.* (2010). We calculated individual biomass using published body length–dry mass equations (Dumont, Van de Velde & Dumont 1975; Smock 1980; Meyer 1989; Benke *et al.* 1999; Baumgärtner & Rothhaupt 2003; Ohta, Miyake & Hiura 2011), except Hydracarina, Ostracoda, Cladocera, Oligochaeta, and Nematoda for which we used biovolume data (Ramsay *et al.* 1997).

Data analysis

All statistical analyses were performed using the programs R (version 2.15.2, R Project for Statistical Computing, Vienna, Austria) and SPSS (version 21.0, IBM Corp., Armonk, New York, U.S.A.). We compared riparian canopy cover at the 2 control and the 6 fire-affected streams over the first 5 years after fire, and between the fire-affected and the control experimental sites from 2003 to 2012 with repeated measures analysis of variance (rmANOVA). We compared mean daily light levels, mean daily water temperature, and other physicochemical variables between the fire-affected and the control sites over the experimental period with 1-way ANOVAs. We log(x)-transformed litter input data (as g AFDM m^{-2} d^{-1}) and analyzed inputs to fire-affected and control sites with an rmANOVA. We compared the abundances of different macroinvertebrate functional feeding groups in 2 control and 6 fire-affected streams over the first 5 postfire years with rmANOVA. For each sampling date, we used the nonparametric Kruskal–Wallis test to identify the times when macroinvertebrate abundance of different FFGs differed significantly between control and fire-affected sites.

We used an exponential decay model (Bärlocher 2005) to quantify leaf breakdown rates:

$$M_t = M_0 e^{-kt} \qquad \text{(Eq. 1.1)}$$

where M_t is the leaf-litter AFDM at time t, M_0 is the initial AFDM corrected for leaching and transportation mass losses, $-k$ is the decomposition rate, and t is the time in days. To test for significant differences in leaf decomposition rates among sites and mesh sizes, we conducted an analysis of covariance (ANCOVA) on $\ln(x)$-transformed AFDM remaining (dependent variable), with site and mesh size as fixed factors and time (in d) as a covariate (Zar 2010). We adjusted α levels for pairwise comparisons of decomposition rates between all sites and mesh sizes with Bonferroni corrections. We corrected for temperature effects on leaf breakdown rates, by repeating the analyses using degree days (in dd) instead of days as the covariate. We calculated degree days as mean daily temperature accumulated by each sampling day (Minshall *et al.* 1983; Irons III *et al.* 1994; Menéndez, Hernández & Comín 2003).

We standardized macroinvertebrate abundance and biomass in coarse-mesh leaf bags per gram of leaf-litter AFDM remaining in leaf bags. We $\log(x + 1)$-transformed macroinvertebrate abundance and analyzed differences between sites with ANCOVA, with site as a fixed factor and time as a covariate. We applied Bonferroni corrections to control for comparison-wise error.

Results

Canopy cover and physicochemical measurements

Canopy cover was lower at fire-affected than at control sites over the first 5 years after fire ($F = 8.2$, $p < 0.04$; Fig. 1.1a) and were consistently lower from 2003–2012 at the fire-affected than at the control experimental sites ($F = 7.1$, $p < 0.02$; Fig. 1.1b). Both sites had alkaline pH, high dissolved oxygen (O_2) levels, low discharge, and low nutrient concentrations during the study period (Table 1.1). Mean daily light levels, mean daily water temperature, and NO_3^--N concentration were substantially higher in the fire-affected than in the control sites (Table 1.1, Fig. 1.2a-b).

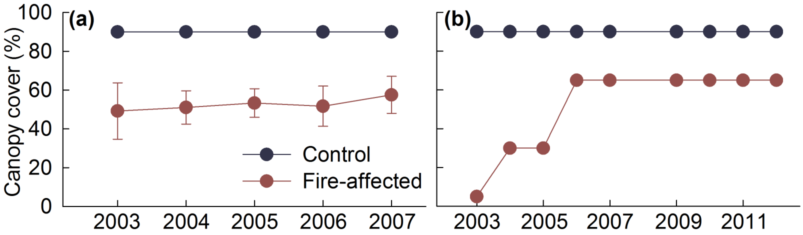


Figure 1.1 Mean (±1 SE) riparian canopy cover at 2 control and 6 fire-affected sites (Verkaik *et al.* 2013b data) over the first 5 years after fire (a) and at the control and the fire-affected sites (this study) from 2003–2012 (b).

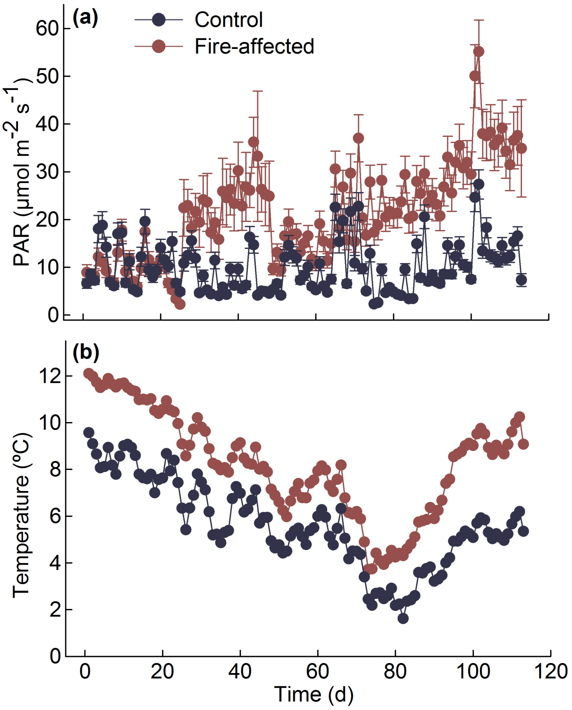


Figure 1.2 Mean (±1 SE, n = 144 measurements d^{-1}) daily light intensity as photosynthetically active radiation (PAR) (a) and water temperature (b) at the control and fire-affected sites during the litter decomposition experiment (23 November 2011–15 March 2012).

Benthic macroinvertebrate communities

The abundances of shredders, scrapers, and gathering collectors peaked in the first year after the fire at fire-affected sites while remaining relatively constant at control sites. After >2 years postfire, the abundances of all functional feeding groups were very similar between control and fire-affected sites (Fig. 1.3a-e). No significant effects of fire on a time × site interaction were detected over the study period (rmANOVA, all $p > 0.05$). In summer 2007, shredders abundances were 523 ± 234 individuals (ind) sample^{-1} at the control sites and 417 ± 101 ind sample^{-1} at the fire-affected sites. These data indicate that the abundances of functional feeding groups were similar between fire-affected and control sites 3 years before the litter decomposition experiment and probably at the time of the experiment.

Table 1.1 Mean (±1 SE) values of characteristics of the control and the fire-affected sites during the litter decomposition experiment (23 November 2011–15 March 2012), and results of analyses of variance comparing characteristics between control and fire-affected sites. PAR = photosynthetically active radiation.

	Control	Fire-affected	*F*	*p*
Elevation (m a.s.l.)	451	485	–	–
Catchment area (ha)	290	510	–	–
Burned area (%)	0	62.1	–	–
Discharge (L s^{-1})	5.5 ± 1.5	18.6 ± 7.7	2.8	0.12
Water temperature (ºC)	5.6 ± 0.2	8.2 ± 0.2	77	<0.001
PAR (µmol m^{-2} s^{-1})	10.1 ± 0.5	21.5 ± 1.0	264	<0.001
pH	8.16 ± 0.02	8.09 ± 0.02	4.0	0.07
Conductivity (µS cm^{-1})	492 ± 2	485 ± 2	4.7	0.06
DO (% saturation)	86.8 ± 0.3	89.3 ± 0.2	4.8	0.09
NH_4^+-N (µg L^{-1})	23 ± 1	24 ± 1	1.7	0.22
NO_3^--N (µg L^{-1})	124 ± 14	640 ± 13	744	<0.001
PO_4^{3-}-P (µg L^{-1})	<10	<10	–	–

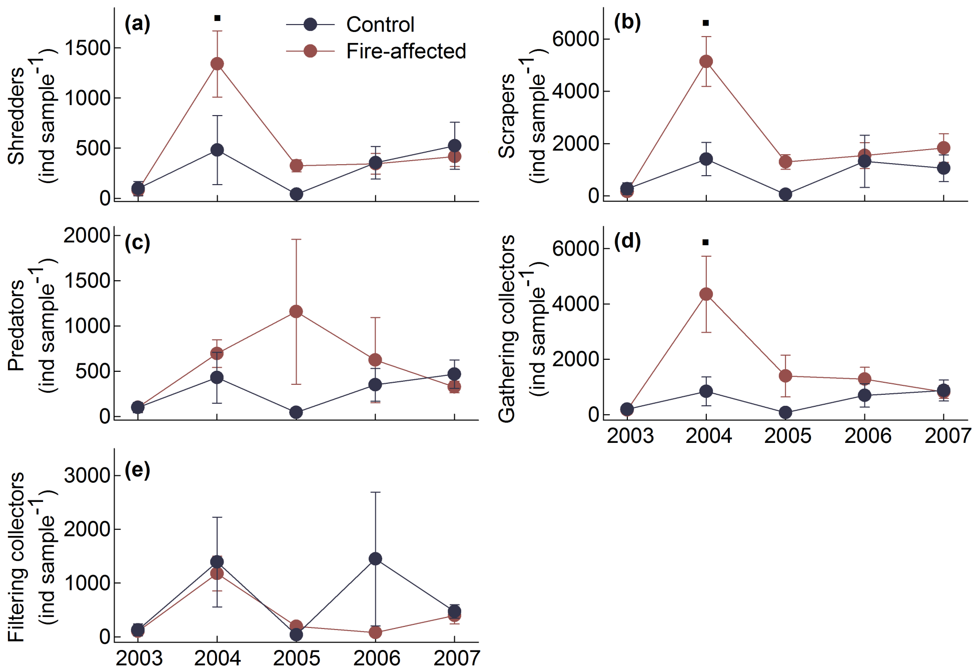


Figure 1.3 Mean (±1 SE) abundances of shredders (a), scrapers (b), predators (c), gathering collectors (d), and filtering collectors (e) at 2 control and 6 fire-affected sites over the first 5 years after fire (data collected by Verkaik *et al.* 2013b). ■ indicates marginally significant difference between control and fire-affected sites on a sampling date ($p < 0.10$).

Litter input

Total litter input from October 2011 to October 2012 was 2.5 times higher at the control (134.6 ± 19.8 g AFDM m^{-2} y^{-1}) than at the fire-affected site (52.6 ± 12.0 g AFDM m^{-2} y^{-1}) ($F = 13.2$, $p < 0.025$; Fig. 1.4a). Leaf inputs were higher at the control than at the fire-affected site ($F = 9.7$, $p < 0.04$; Fig. 1.4b), but inputs of bark, twigs, fruits, or flowers did not differ between sites. Both total litter and leaf inputs were affected by a site × sampling date interaction (total litter: $F = 2.2$, $p < 0.03$; leaf input: $F = 4.5$, $p < 0.001$), with leaf inputs peaking from late spring to early autumn in the control site but remaining at low, constant levels in the fire-affected site (Fig. 1.4b). Leaf-litter made up 62% of the total annual litter input and consisted primarily of leaves of *Q. ilex*, *Q. robur*, *P. alba*, *Viburnum* sp., *Salix* sp., *P. halepensis*, and *Fraxinus* sp.

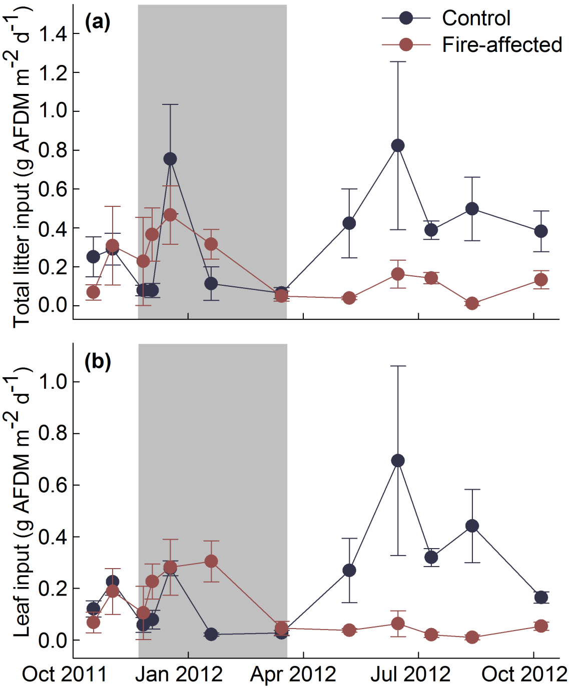


Figure 1.4 Mean (±1 SE; n = 3 replicate litter traps) total litter (a) and leaf (b) inputs at control and fire-affected sites from 4 October 2011 to 4 October 2012. Grey area indicates the time period when the leaf decomposition experiment was done.

Leaf-litter decomposition experiment

The loss of poplar leaf mass was well fit by an exponential model, with a slightly better fit to degree days than days (R^2 higher in all 4 cases; Table 1.2). Litter mass loss over time without temperature correction differed between sites ($F = 63$, $p < 0.001$) and mesh sizes ($F = 26$, $p < 0.001$), and was faster at the fire-affected than at the control site, and in coarse- than in fine-mesh bags (Table 1.2, Fig. 1.5a). Using degree days instead of days as the independent variable, leaf breakdown rates also differed between sites ($F = 10.5$, $p = 0.002$) and mesh sizes ($F = 48$, $p < 0.001$). However, pairwise comparisons showed mass loss differences between sites for coarse-mesh bags ($F = 19$, $p < 0.001$) but not for fine-mesh bags ($F = 1.9$, $p = 0.18$; Fig. 1.5b). Litter-loss rates were greater in coarse- than fine-mesh bags at both sites (control: $F = 23$, $p < 0.001$; fire-affected: $F = 30$, $p < 0.001$; Fig. 1.5b).

Table 1.2 Mean (SE) leaf-litter breakdown rates (–*k*) and R^2 values for regressions of ln(*x*)-transformed leaf mass remaining vs time in days (d) and cumulative degree days (dd).

		d			dd		
Site	Mesh	–*k*	SE	R^2	–*k*	SE	R^2
Control	Coarse	0.00445	0.00025	0.92	0.00084	0.000032	0.96
Control	Fine	0.00370	0.00019	0.93	0.00069	0.000026	0.96
Fire	Coarse	0.00774	0.00036	0.94	0.00098	0.000034	0.97
Fire	Fine	0.00484	0.00038	0.85	0.00062	0.000040	0.90

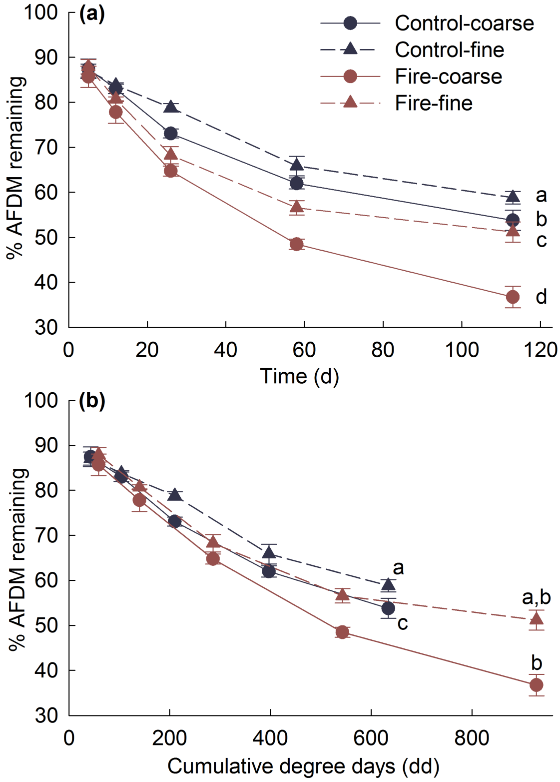


Figure 1.5 Mean (±1 SE; *n* = 6 replicate leaf bags) % initial *Populus alba* leaf-litter mass remaining in coarse- and fine-mesh bags at the control and the fire-affected sites over 113 days expressed over time (d) (a) and over cumulative degree days (dd) (b). Curves with the same letter do not have significantly different decomposition rates ($p < 0.05$).

Across all coarse-mesh leaf bags, total macroinvertebrate density varied from 1 to 127 and shredder density from 0 to 76 ind g^{-1} leaf-litter AFDM. Total macroinvertebrate, shredder, scraper, and predator abundances in coarse-mesh bags were higher at the fire-affected than at the control site ($F = 9 – 16$, $p < 0.005$ to < 0.001), but the abundances of gathering and filtering collectors did not differ between sites. The biomass of total macroinvertebrates ($F = 4.8$, $p < 0.04$; Fig. 1.6a) and shredders ($F = 4.4$, $p < 0.04$; Fig. 1.6b) in coarse-mesh bags were higher at the fire-affected than at the control site, but no intersite differences were detected for the biomass of other functional feeding groups (Fig. 1.6c-f).

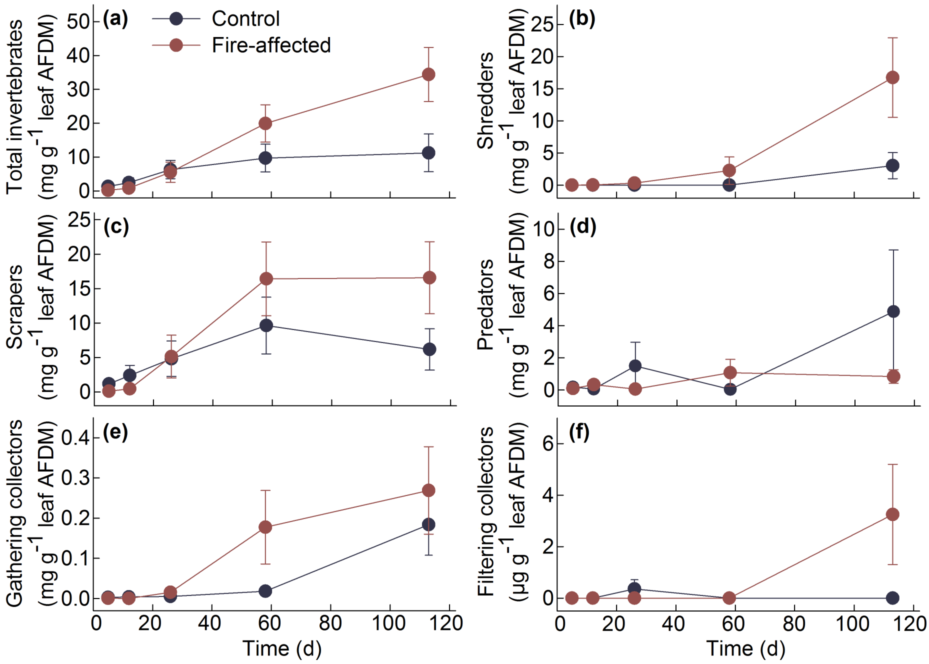


Figure 1.6 Mean (±1 SE; $n = 6$ replicate bags) biomass (mg g^{-1} remaining leaf ash-free dry mass [AFDM]) of total invertebrates (a), shredders (b), scrapers (d), predators (d), gathering collectors (e), and filtering collectors (f) in coarse-mesh bags at the control and the fire-affected streams over the experimental period.

Discussion

In a recent review of wildfire effects on Mediterranean streams, Verkaik *et al.* (2013a) concluded that algal, invertebrate, and amphibian communities recover faster after fire in Mediterranean streams than in streams located in other climatic regions: 1 to 4 years in Mediterranean streams vs 5 to 10 years in nonMediterranean streams. However, our study indicates that the legacy effects of wildfire accelerated leaf-litter breakdown in a Mediterranean stream for up to 8 years after fire. We also found that wildfire reduced and altered the timing of allochthonous organic matter inputs into a stream after 8 years postfire. Despite the high resilience to fire of biological communities in Mediterranean streams, our study suggests that past wildfires in Mediterranean streams can have long-term consequences for stream ecosystem processes. The leaf-litter decomposition component of our study was based on only 2 streams (fire-affected vs control), but additional data and observations from these and other sites and times indicate that these 2 streams were similar before fire affected one of them and that the proposed mechanisms responsible for our results (see below) are consistent with this information. Thus, we treat our study as an example of how wildfire can trigger long-term changes in ecosystem processes in Mediterranean streams. Our study also illustrates how landscape history, a factor often overlooked in stream ecology, can influence the functioning of lotic ecosystems.

Our results show, as we expected, that opening of the riparian forest canopy by fire increased site light levels and, consequently, water temperature (Gresswell 1999; Koetsier *et al.* 2010). Wildfire also reduced annual litter inputs, primarily leaf inputs, by 2.5 times after 8 years postfire. Leaf inputs peaked from late spring to early autumn in the control site, consistent with the results of Fioretto *et al.* (2003) for a low Mediterranean shrubland stream and with the contention of Gasith and Resh (1999) and González (2012) that litterfall should be more protracted in Mediterranean than in temperate streams, which have a marked autumnal peak. In contrast, litter inputs to the fire-affected site were lower and more constant, indicating that wildfire affected both the quantity and timing of litter inputs (Jackson *et al.* 2012).

Leaf breakdown rates were low but similar to published data for *P. alba* leaves during autumn–winter in Mediterranean rivers (Menéndez *et al.* 2003). Litter breakdown rates without temperature correction were faster at the fire-affected than at the control site in both fine- and coarse-mesh bags. However, fine-mesh decomposition rates did not differ between sites after temperature correction, indicating that microbially mediated decomposition differences between sites were driven almost entirely by differences in temperature. Consistent with our hypothesis, the removal of riparian cover by fire apparently led to higher water temperatures, which increased microbial activity and litter decomposition in the fire-affected site, consistent with literature data indicating the primacy of temperature in determining litter decomposition rates (Webster & Benfield 1986; Short & Smith 1989; Gessner, Robinson & Ward 1998; Ferreira & Chauvet 2011a). Despite the low nutrient concentrations recorded in our study sites, NO_3^--N concentrations were >5 times higher in the fire-affected than in the control site, which may have contributed to accelerate microbial leaf decomposition at the fire-affected site (Benstead *et al.* 2009; Ferreira & Chauvet 2011b; Woodward *et al.* 2012). Nevertheless, the lack of difference in decomposition rates in fine-mesh bags between sites when expressed as a function of degree days suggests that temperature was the primary factor driving microbial decomposition rates.

Leaf breakdown was faster in coarse- than fine-mesh bags in both sites, and overall decomposition was faster in the fire-affected than in the control site. These differences persisted even after temperature corrections. Thus, differences in total decomposition rates between sites should be caused by factors additional to temperature (McArthur *et al.* 1988). Physical fragmentation and abrasion of leaves were unlikely to be important for leaf breakdown in our study because leaves were deployed in pools at water velocities <0.15 m s^{-1}, far below threshold velocities reported to affect leaf breakdown rates (Ferreira *et al.* 2006a). Moreover, there was no evidence of the mechanical fragmentation of leaves. Sediment deposition was unlikely to be important to leaf breakdown because no differences in substrata characteristics were discernible between sites, and burial of leaf bags by sediment was not observed. After dismissing the effects of possible differences in current velocity, mechanical fragmentation, and sediment deposition on leaf decomposition between bags with different mesh sizes, we isolated the effects of shredders on leaf breakdown rates by subtracting *k*-values in fine-mesh bags from those in coarse-mesh bags. These calculations showed that shredder-mediated leaf breakdown rates were 3.9 times higher at the fire-affected than at the control site (0.0029 vs 0.00075) when expressed as days^{-1}, and 2.4 times higher when expressed as degree days^{-1} (0.00036 vs 0.00015). This difference in shredder-mediated leaf breakdown rates between study sites could be attributed to intersite differences in shredder densities in coarse-mesh bags, which were significantly higher in the fire-affected than in the control site by the end of the experiment. Therefore, faster overall leaf decomposition in the fire-affected than in the control site probably was caused by both higher microbial activity, driven by higher temperatures and higher shredder activity in coarse-mesh bags at the fire-affected site.

Higher shredder abundance and biomass in coarse-mesh bags at the fire-affected site were not driven by differences in shredder abundance in the benthos of fire-affected vs control sites because benthic invertebrate abundances recovered quickly after fire, and shredder densities were similar between sites affected and unaffected by fire within 3 years postfire. Moreover, it is unlikely that shredder aggregation in the leaf bags was driven by refuge responses to predators (Lagrue *et al.* 2011) because vertebrate predators were absent at both sites and invertebrate predators could move freely into and out of coarse-mesh bags. On the other hand, a variety of studies indicate that shredders aggregate in resource patches when litter availability is low, thereby accelerating litter breakdown (Baldy & Gessner 1997; Rowe & Richardson 2001; Tiegs *et al.* 2008). We did not measure in-stream CPOM levels, but lower litter inputs to the fire-affected than to the control sites from May to September probably led to lower in-stream CPOM levels from November to March, when the leaf bag experiment was done. Our results show that *Populus alba* leaf decomposition rates were slow, with leaves lasting several months before being totally skeletonized (e.g. ~54% of the leaf mass remained in control leaf bags after nearly 4 months in autumn–winter; Fig. 1.5a-b).

Moreover, wildfires may reduce retention of benthic CPOM in streams by altering the characteristics and inputs of woody debris and removing debris dams via postfire flooding (Vieira *et al.* 2011, Vaz *et al.* 2013). A year after a fire in southern California, CPOM levels were lower in fire-affected than in reference sites, but in subsequent years, CPOM levels became similar in streams draining burned vs unburned catchments where riparian vegetation remained intact, but remained lower at sites where riparian vegetation burned (Cooper *et al.* 2015). These results suggest that wildfire effects on CPOM availability depend on the condition of the riparian forest. In addition, higher light levels and NO_3^--N concentration in the fire-affected site could have increased algal growth on leaves, and increased NO_3^--N concentration could have increased leaf N content, with both enhancing litter quality and attractiveness to shredding invertebrates (Robinson & Gessner 2000; Lagrue *et al.* 2011; Tant, Rosemond & First 2013). Therefore, shredder aggregation in leaf bags at the fire-affected site could be attributed to resource tracking by shredders where leaf-litter inputs and probably benthic levels were low and, perhaps, where leaf palatability was high.

The observed fast recovery of shredder abundances after fire despite reductions in litter inputs suggests a weak relationship between litter inputs and shredder density (Linklater 1995), in contrast to studies showing positive relationships between leaf-litter inputs or availability and shredder density (Richardson 1991; Dobson & Hildrew 1992; Boulton & Lake 1992b; Friberg 1997; González & Graça 2005). Recovery of shredder abundances after wildfire might be explained by shredder dietary shifts to available secondary resources when leaf-litter becomes scarce (Mihuc & Minshall 1995; Costantini & Rossi 1998; Graça 2001; Dangles 2002), such as after a wildfire. Authors of several stable-isotope studies have reported a postfire shift from a detritus-based to a periphyton-based food web in streams (Spencer, Gabel & Hauer 2003; Mihuc & Minshall 2005; Cooper *et al.* 2015). Compared to temperate streams, Mediterranean streams have more macroinvertebrates with traits favoring dispersion and rapid recolonization of disturbed streams, such as aerial active dispersal, more frequent reproduction, and small size, which can explain the fast recovery of macroinvertebrate abundances after fire (Bonada, Dolédec & Statzner 2007a; Verkaik *et al.* 2013b). In fact, 5 years after fire, only a few individual taxa with low mobility and limited dispersal capacity were still less abundant in fire-affected than in control sites (Verkaik *et al.* 2013b). The observed peak in the abundances of shredders, scrapers, and gathering collectors in the fire-affected sites in the first year after fire may be caused by a short-term increase in resource availability. Britton (1990) reported an increase in leaf-litter input to streams immediately after fire but a decrease over the following years, and Cooper *et al.* (2015) showed that in-stream algal production where riparian vegetation was burned increased after the first postfire wet period apparently because of increased light levels and high nutrient concentrations, but declined afterward despite the high light levels.

In conclusion, our study shows that a wildfire that occurred 8 years ago accelerated leaf-litter decomposition in a Mediterranean stream because of increased microbial activity associated with higher water temperatures engendered by removal of canopy cover and by shredder aggregation in leaf packs, probably because of scarcity or high palatability of leaf-litter in the fire-affected stream. These results indicate that wildfire effects on the riparian forest are key factors mediating wildfire effects on streams, as suggested in previous studies (Arkle & Pilliod 2010; Verkaik *et al.* 2013a; Cooper *et al.* 2015). More research on the effects of wildfires on riparian–stream linkages and ecosystem processes is needed to develop effective management practices, given the general lack of scientific studies on these topics and projected increases in wildfire risk.

Chapter 2

Small but powerful: top predator local extinction affects ecosystem structure and function in an intermittent stream

Top predator loss is a major global problem, with a current trend in biodiversity loss towards high trophic levels that modifies most ecosystems worldwide. Most research in this area is focused in large-bodied predators, despite of the high extinction risk of small-bodied freshwater fish that often act as apex consumers. The aim of our research was to determine how this global problem affects intermittent streams and, in particular, if the loss of a small-bodied top predator (1) leads to a ‘mesopredator release’, affects primary consumers, and changes whole community structure and (2) triggers a cascade effect modifying the ecosystem function. To address these questions, we studied the top-down effects of a small endangered fish species, *Barbus meridionalis* (the Mediterranean barbel), conducting a mesocosm experiment in an intermittent stream where *B. meridionalis* became locally extinct following a wildfire. We found that top predator absence led to ‘mesopredator release’, and also to ‘prey release’ despite intraguild predation, which contrasts with traditional food web theory. In addition, *B. meridionalis* extirpation changed whole macroinvertebrate community composition and increased total macroinvertebrate density. Regarding ecosystem function, periphyton primary production decreased in apex consumer absence. In this study, the apex consumer was functionally irreplaceable, its local extinction led to the loss of an important functional role that resulted in major changes to the ecosystem’s structure and function. This study evidences that intermittent streams can be affected by the consequences of apex consumers’ extinctions, and that the loss of small-bodied top predators can lead to large ecosystem changes.

Introduction

Predation is an important species interaction that has implications for biological populations, communities, and ecosystems. In addition to affecting prey abundance and distribution, predation affects other non-prey taxa and ecosystem processes through indirect pathways (Gurevitch, Morrison & Hedges 2000; Holomuzki, Feminella & Power 2010). In recent decades, human activity has caused the extinction of many apex consumers (i.e. top predators) (Duffy 2002; Estes *et al.* 2011) and several studies have indicated subsequent ecosystem changes that are complex, unpredictable, and largely unknown (Marshall & Essington 2011; Estes *et al.* 2011). Given that current biodiversity loss is biased towards species in the higher trophic levels (Duffy 2002; Schneider & Brose 2013), the ecosystem impacts of top-predator decline remain a research priority (Sutherland *et al.* 2013).

The extinction of top predators is often associated with an increase in mesopredators (Ritchie & Johnson 2009; Prugh *et al.* 2009; Brashares *et al.* 2010), i.e. any mid-ranking predator in a food web. An ecosystem may have several mesopredators, and a mesopredator in one system may be a top predator in another system (Prugh *et al.* 2009). 'Mesopredator release' often leads to a decrease in the prey (Ritchie & Johnson 2009; Brashares *et al.* 2010), a straightforward conclusion, termed a trophic cascade, when each trophic level is connected in a direct and negative way (Carpenter, Kitchell & Hodgson 1985; Henke & Bryant 1999; Ritchie & Johnson 2009). But, as showed in a recent review about apex-mesopredator-prey interactions (Brashares *et al.* 2010), not all trophic webs have a linear shape. From the 32 studies, Brashares *et al.* (2010) found that 40% of the interactions were triangular: those in which top predators feed on mesopredators and also on prey, resulting in intraguild predation (IGP; characterized by predators that feed on other predators with which they share prey taxa). If IGP occurs, the apex consumer exerts top-down control on both mesopredator and prey, and then, apex consumer extinction would liberate mesopredator and prey from its top-down structuring forces. However, in that case, 'mesopredator release' could also lead to an increase on prey top-down control, neutralizing apex consumer loss. This would result in a negative or a null net effect on prey taxa and, consequently, dampen the trophic cascade on primary production (Polis, Myers & Holt 1989; Rosenheim *et al.* 1995; Finke & Denno 2005). In addition, according to the predator-mediated coexistence theory (Caswell 1978) and to recent modelling work (Petchey *et al.* 2004), apex consumer loss can cause secondary extinctions in adjacent and non-adjacent trophic levels (Henke & Bryant 1999; Borrvall & Ebenman 2006; Säterberg *et al.* 2013), mainly because predators can facilitate coexistence among prey species. Thus, top predator extinctions have been related not only to an increase in mesopredator abundance, but also to a decline in biodiversity (Henke & Bryant 1999; Ritchie & Johnson 2009).

Intermittent streams are present in all climate areas and are ecologically unique (Larned *et al.* 2010; Nikolaidis *et al.* 2013), but most research in these systems focused on how hydrological variability shapes community attributes and biogeochemical processes (Larned *et al.* 2010; García-Roger *et al.* 2011), while the role of top-down structuring forces has been largely neglected. Furthermore, intermittent streams often lack large aquatic consumers that are often considered to be top predators, and instead, are typically inhabited by predaceous invertebrates and small-bodied fish (Meyer *et al.* 2007; Reich *et al.* 2010). These systems have been considered a refuge from vertebrate predation (Williams 1996; Meyer *et al.* 2007), and even from invertebrate predation, as some studies suggest predatory invertebrates have lower abundances in intermittent than in permanent streams (Bogan *et al.* 2013). Other research evidence indicates that predation pressure increases with stream fragmentation in isolated pools, typically in summer, when predatory lentic invertebrates (odonates, hemipterans, and coleopterans) replace reophilous taxa (Boulton & Lake 1992a; Stanley *et al.* 1994; Rieradevall *et al.* 1999; Bonada *et al.* 2007b). Regarding predatory vertebrates, previous studies of intermittent streams show that predatory fish can affect stream macroinvertebrates in terms of: whole community assemblage and total density (Williams *et al.* 2003), the densities of specific groups (e.g. air breathing macroinvertebrates (Closs 1996)), total biomass (Wesner 2013), and prey body condition (Love *et al.* 2005). Conversely, other studies suggest that predatory fish have no effect on macroinvertebrate communities (Ludlam & Magoulick 2010). All these studies were performed in dry season conditions, in isolated pools or in pools that became isolated during the experiment, when predation pressure reaches its peak in these systems. The importance of predation in intermittent streams during periods of flow remains unknown.

The objective of our research was to determine if the loss of an endangered apex consumer from an intermittent stream would result in major changes to ecosystem structure and function. *Barbus meridionalis* (A. Risso, 1827), also known as the Mediterranean barbel, is an endemic small-bodied fish of Spain and France that often act as apex consumer in intermittent streams. This species is considered 'vulnerable' in the Spanish Red Book and 'near threatened' internationally. We studied the top-down impacts of *B. meridionalis* to determine if the loss of the top predator (1) leads to a 'mesopredator release', affecting primary consumers and changing whole community structure, and (2) triggers a cascade effect modifying ecosystem function (i.e. periphyton primary production). *Barbus meridionalis* has been classified as an insectivore benthic species (Benejam *et al.* 2010) that feeds primarily on chironomid larvae, detritus (which could be explained by its benthic feeding behavior), mayflies, and isopods (mainly primary consumers (Mas-Martí *et al.* 2010)). Thus, apex consumer extirpation might not lead to 'mesopredator release', and instead could promote a trophic cascade resulting in 'prey release' and lower primary production (i.e. 'prey release' hypothesis, see Fig. 2.1a). Alternatively, *B. meridionalis* could feed on two trophic levels (i.e. macroinvertebrate secondary and primary consumers), in which case top predator removal would trigger a 'mesopredator release' due to IGP. According to IGP theory, 'mesopredator release' could compensate apex consumer extirpation in terms of prey top-down control, and the trophic cascade would be dampened with no impact on prey or primary production (i.e. 'mesopredator release' hypothesis, see Fig. 2.1b). To address these questions, we performed a field experiment using enclosure/exclosure mesocosms in a Mediterranean stream where *B. meridionalis* became locally extinct following a wildfire.

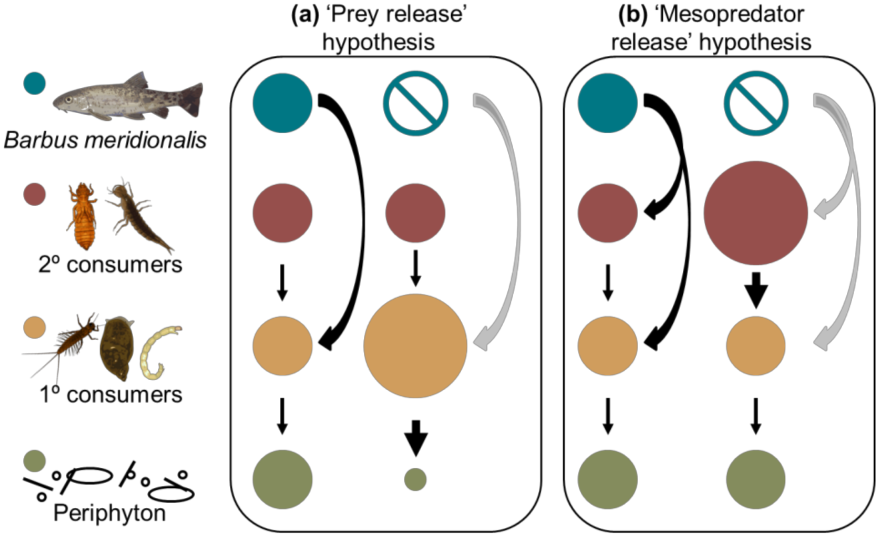


Figure 2.1 Diagram of the trophic interactions in intermittent stream food webs in the presence and absence of the apex consumer. This diagram describes our two hypotheses related to apex consumer extirpation: 'prey release' hypothesis (a) and 'mesopredator release' hypothesis (b). Circumference size in top predator absence diagrams represents the density decrease, increase or persistence compared to the top predator presence diagram. Arrows represent trophic interactions. Thicker arrows = magnified trophic interactions due to apex consumer extirpation; grey arrows = lost trophic interactions after apex consumer extirpation.

Materials and methods

Study area

The Vall d'Horta stream (41°40'24''N, 2°02'4''E; Altitude: 480 m a.s.l.) is a first order stream located in the protected area of Sant Llorenç del Munt i l'Obac Natural Park (50 km inland from Barcelona, NE Spain). The main stream course is formed from the confluence of the Pregona and Font del Llor creeks draining to the Ripoll's Basin (a tributary of the Besòs River). This hilly area is characterized by a Mediterranean climate and a calcareous geology, with alternating highly permeable and less permeable substrates where springs are located (see Rieradevall *et al.* 1999 and Verkaik *et al.* 2013b for a detailed site description). *Barbus meridionalis* is a common fish within these intermittent streams that find refuge in the remaining permanent pools during periods of hydrological disconnection (usually in summer). In August 2003, a wildfire burned a forested area of 4543 ha, affecting 62% of the Vall d'Horta basin. As a consequence of this wildfire, *B. meridionalis* became locally extinct in some of the affected streams, even in the pools, potentially due to chemical changes that occurred during the first rainfall events (Vila-Escalé *et al.* 2007a). The fish population has not recovered since the fire, most likely due to natural and human barriers in the lower part of the study site.

We conducted the experiment in a 100-m reach formed by a large pool where riparian vegetation was not burned by the wildfire. This reach was selected because, as observed in the years before the fire, barbels took refuge in these pools to survive periodic drought conditions present in the area when intermittent Mediterranean streams were reduced to isolated pools (Vila-Gispert & Moreno-Amich 2001). Physicochemical water analyses (n = 9) were performed before, during, and at the end of the experiment. The results (presented as the mean ± SE) confirmed that water of this reference stream was hard (conductivity: 520 ± 5 μS cm^{-1}; pH: 7.9 ± 0.1) and oligotrophic (N-NO_3^{-1}: 290 ± 20 μg L^{-1}; N-NH_4^+: 19 ± 3 μg L^{-1}; P-PO_4^{-3} <10 μg L^{-1}). The stream discharge averaged 15.7 ± 0.9 L s^{-1}, which, with the very low water velocity in the pool (<1 cm s^{-1}), naturally kept the pool water renewed and oxygenated (DO_2: 9.6 mg L^{-1}, 84.7%) during our study.

Mesocosm design

We performed an enclosure/exclosure mesocosm experiment to manipulate *B. meridionalis* densities. Removal experiments that simulate the loss of one or more species from a natural community can reveal the consequences of apex consumer extinctions and assess biodiversity-ecosystem function (B-EF) relationships (Diaz *et al.* 2003).

We used nine large cages (100 x 100 cm surface, 70 cm height; see Fig. 2.2) covered with a 10-mm mesh that retained fish but allowed macroinvertebrate emigration/immigration, thereby minimizing the impact of our experimental design on the rate of prey exchange with the benthos

(Cooper, Walde & Peckarsky 1990; Allan & Castillo 2007). In each cage, four plastic trays (40 x 40 cm surface, bottom of 1-mm mesh size) were used as replicates (36 trays in total); each tray contained four medium-sized stones for macroinvertebrate colonization and three glass tiles (2 x 4 cm) for periphyton colonization (see Fig. 2.2). Tray substrates within the mesocosms were complex due to the material deposited during the colonization period; substrate was formed by a mixture of sediment, detritus, and leaves, which provided some refuge to invertebrates (Reice 1991; Rosenfeld 2000) along with the initial added stones. To study the consequences of *B. meridionalis* extirpation, we tested three treatments with varying barbel density levels in the enclosures: i) no fish; ii) barbels at low density (i.e. 2 individuals m^{-2}, the known prefire density; A. de Sostoa pers. comm.); and iii) barbels at high density (i.e. 4 individuals m^{-2}, two fold the prefire density). Barbels were caught using an electrofishing source downstream from our study site, and individuals selected for the experiment were approximately the same size (total length 101.8 ± 2.6 mm; mean ± SE) and weight (2.3 ± 0.2 g). To ensure similar initial conditions, barbels were kept in observation for 24 h before starting the experiment after electrofishing and transportation.

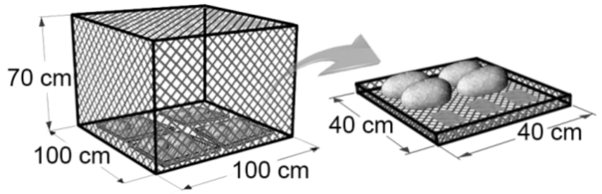


Figure 2.2 Diagram of the experimental enclosure and one of the four identical trays that contained stones for macroinvertebrate colonization and glass tiles for periphyton colonization. Dimensions are indicated.

Sampling and laboratory protocols

The field experiment was conducted in late spring of 2010 before pool disconnection (flow averaged 15.7 ± 0.9 L s^{-1}), over the course of five weeks. Three weeks were allowed for periphyton and macroinvertebrate colonization, a time previously described as adequate for equilibrating the mesocosm and background macroinvertebrate densities (Lancaster, Hildrew & Townsend 1991). Two weeks were allowed for barbel interaction. During the colonization period, the cage tops were opened to facilitate aerial colonization and the entrance of organic material. Before the addition of barbels to the experimental enclosures, one tray per cage (n = 9) was removed and sampled to test if there were differences in colonization among cages. Barbel density levels were randomly assigned to enclosures, and the cage tops were closed following barbel introductions to avoid bird or mammal predation. After two weeks of interaction, mesocosms were destructively sampled with the same effort for each tray (n = 27; 9 trays per treatment). Tray contents (with stones) were carefully washed in a 250-µm mesh sieve and preserved in 4% formalin until being processed in the laboratory. All samples were sorted, counted, and identified. Taxonomic resolution was primarily to the genus level, including Chironomidae. Some Diptera were identified to the family level, and Oligochaeta, Ostracoda, Cladocera, Copepoda, Hydracarina, and terrestrial invertebrates identified to higher levels. Each taxon was categorized as either secondary or primary consumer according to Merritt & Cummins (1996) and Tachet *et al.* (2010). Periphyton net primary production was measured as the net accumulation of chlorophyll-*a* on artificial substrata (Godwin & Carrick 2008). Chlorophyll-*a* was measured after extraction in acetone (90%) for 24 h in the dark at 4°C, sonication for 5 minutes at 40 kHz, and filtration (GF/F Whatman 0.7-µm pore size). Following Jeffrey & Humphrey (1975), chlorophyll-*a* concentration was determined spectrophotometrically (Perkin-Elmer, Lambda UV/VIS).

In order to test if *B. meridionalis* also feeds on predatory invertebrates (not only on primary consumers), and therefore, if intraguild predation occurs, we analyzed barbels' gut contents. Barbels were euthanized using an overdose of anaesthetic (MS-222). Gut contents were preserved in 4% formalin, sorted, counted, and identified at the same taxonomic level as the benthic samples.

Data analysis

To test differences among the three barbel density treatments, we used the non-parametric Kruskal-Wallis test (K-W test). Then, pairwise Mann-Whitney *U*-tests were used to detect significant differences between treatments. We compared total macroinvertebrate abundance (total number of individuals m^{-2}), taxa richness (number of different taxa), rarefied taxa richness (taxa richness corrected by macroinvetebrate abundance in the sample), Simpson's diversity index (*D*, calculated as $D=\sum_i(n_i(n_i-1)/N(N-1))$, where n_i is the number of individuals of taxon i and N is the total number of macroinvertebrates (Hurlbert 1971)), abundance of common taxa (number of individuals of each abundant taxon, i.e. >50 ind m^{-2} in the treatment lacking barbels), and periphyton net primary production (net accumulation of chlorophyll-*a*) among the three treatments.

We used permutational multivariate analysis of variance (PERMANOVA, 'Adonis' function in R) on the Bray-Curtis distance matrix, after the log-transformation of the macroinvertebrate abundance data, to test differences in macroinvertebrate community composition among treatments. Afterwards, we used indicator species analysis, using 'IndVal' test in R, to identify which taxa of the macroinvertebrate communities could serve as indicator for each barbel density treatment. The 'IndVal' test calculated the indicator value for each taxon, combining measurements of taxon specificity to each established barbel density treatment with taxon fidelity within each treatment (Dufrêne & Legendre 1997). The significance of 'IndVal' measures was tested using the Monte Carlo test with 9999 permutations.

We also calculated predator:prey ratios for abundance and richness, dividing the abundance (or richness) of secondary consumers by that of primary consumers for each sample. To test for intraguild predation, we also categorized each taxon found in the gut contents as either primary or secondary consumer, and calculated the proportion (%) of each category in the contents. All statistical analyses were performed in R 2.15.2., we used 'vegan' and 'labdsv' packages (R Core Team 2012).

Ethical note

This study was authorized by the Autonomous Government of Catalonia (Generalitat de Catalunya) and the Natural Parks Department of the Government of Barcelona (Diputació de Barcelona). The University of

Barcelona reviewed and approved the project without requirement for ethics approval. Fish were euthanized following the standard protocol recommended by the animal welfare service at the University of Barcelona (anaesthetized using Tricaine methanesulfonate (MS-222)), and all efforts were made to minimize animal stress and suffering during this study.

Results

We found 81 taxa (76 aquatic invertebrates, 1 amphibian, and 4 terrestrial invertebrates) throughout the mesocosm experiment. Macroinvertebrate communities in the mesocosm were similar to those found during previous research in the stream (Verkaik *et al.* 2013b). Primary consumers were typically chironomids, mayflies (such as *Habroplebia* sp., *Baetis* sp., or *Caenis* sp.), gastropods (such as *Gyraulus* sp. or *Radix* sp.), and crustaceans (Cladocera and Ostracoda); while secondary consumers were dominated by predatory chironomids (*Zavrelimyia* sp. and *Procladius* sp.), water beetles (mainly from Dytiscidae family), hemipterans (*Parasigara* sp.), Odonates (such as *Chalcolestes viridis*, *Sympetrum* sp., or *Aeshna* sp.), and leeches (*Helobdella stagnalis*) (Table S.1). Community-level analyses of the macroinvertebrate samples before the addition of barbels to the enclosures showed a homogeneous colonization of the experimental cages. Total macroinvertebrate density, taxa richness, Simpson's diversity index, and community composition did not differ among cages (K-W tests, $p > 0.1$; Adonis, $F = 0.69$, $p = 0.87$). Similarly, significant differences in periphyton net primary production were not observed (K-W test, $\chi^2 = 0.39$, $p = 0.83$).

Barbel presence reduced macroinvertebrate total density ($\chi^2 = 9.09$, $p = 0.011$); macroinvertebrate density declined almost by half (46.2%) in the treatment with high barbel density compared to the treatment that did not contain barbels ($U = 12$, $p = 0.01$). We did not detect significant differences among treatments in taxa richness ($\chi^2 = 4.29$, $p = 0.12$) or in the Simpson's diversity index ($\chi^2 = 0.77$, $p = 0.68$). The density of the most abundant macroinvertebrate taxa declined when barbels were present, but vulnerability varied among prey (Fig. 2.3, Table S.1). We distinguished four patterns of abundance related to barbel density: i) a decrease in abundance proportional to barbel density for some taxa such as *Habrophlebia* sp. and *Chalcolestes viridis* (see Fig. 2.3c,f); ii) a sharp decrease in abundance at barbel presence (i.e. at both low and high barbel densities, but not proportional to barbel presence) for other taxa (e.g. mobile predators *Stictonectes* sp. and *Chaoborus* sp.; see Fig. 2.3g-h); iii) a significant reduction in taxa abundance only at high barbel density treatment compared to the other treatments (e.g. *Zavrelimyia* sp.; see Fig. 2.3b); and iv) no change in abundance for other taxa irrespective of barbel densities (e.g. *Gyraulus* sp.; see Fig. 2.3d).

There were significant differences among the three treatments in the composition of macroinvertebrate communities (Adonis, F = 2.39, $p < 0.001$). Twelve taxa were identified as indicators in the treatment that did not contain barbels (Table 2.1) and two taxa in the low barbel density treatment. No indicator taxa were found in the high barbel density treatment.

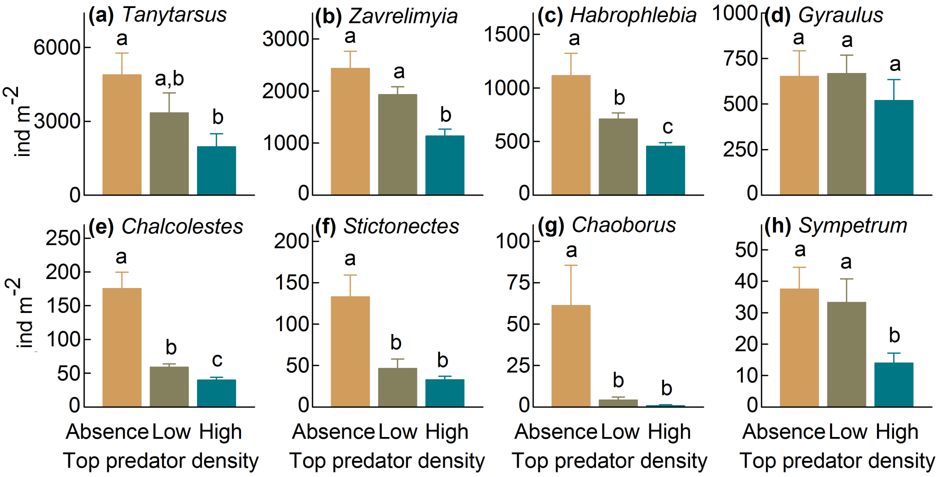


Figure 2.3 Macroinvertebrate abundance for eight of the most abundant taxa (>50 ind m^{-2} in the treatment lacking barbels) in the three treatments with varying *B. meridionalis* densities. Bars represent mean ± SE (individuals m^{-2}). Graphs are sorted by taxa abundance: *Tanytarsus* sp. (a), *Zavrelimyia* sp. (b), *Habrophlebia* sp. (c), *Gyraulus* sp. (d), *Radix* sp. (e), *Chalcolestes viridis* (f), *Stictonectes* sp. (g), and *Chaoborus* sp. (h). Different letters correspond to significant differences resulting from the pairwise comparisons among treatments (*U*-test, $p < 0.05$).

Table 2.1 Macroinvertebrate taxa detected as significant indicators for the three barbel density treatments. T – Treatments: 1 = treatment whithout barbels, 2 = treatment with a low density of barbels. IndVal – indicator value. *p* – its respective *p*-value.

Taxa	T	IndVal	*p*
Chaoborus sp.	1	72.05	<0.001
Cloeon sp.	1	70.88	<0.001
Parasigara sp.	1	69.02	<0.001
Procladius sp.	1	65.10	0.008
Chalcolestes viridis	1	64.04	<0.001
Agabus sp.	1	63.40	0.010
Stictonectes sp.	1	62.69	<0.001
Ostracoda	1	56.53	0.002
Cladocera	1	55.97	0.010
Radix sp.	1	53.33	0.019
Habrophlebia sp.	1	48.90	<0.001
Zavrelimyia sp.	1	44.30	0.012
Oulimnius sp.	2	56.56	0.007
Copepoda	2	49.97	0.021

When we analyzed macroinvertebrate communities separately for primary and secondary consumers, we detected that *B. meridionalis* density affected primary consumer abundance ($\chi^2 = 7.38$, $p = 0.025$; Fig. 2.4a) but not primary consumer richness ($\chi^2 = 1.19$, $p = 0.55$) or rarefied richness ($\chi^2 = 1.42$, $p = 0.49$; Fig. 2.4b). Top predator absence increased secondary consumer abundance ($\chi^2 = 12.49$, $p = 0.002$; Fig. 2.4c) and richness before ($\chi^2 = 12.89$, $p = 0.002$) and after rarefaction ($\chi^2 = 8.17$, $p = 0.017$; Fig. 2.4d). The ratio for predator:prey abundance marginally increased (abundance: $\chi^2 = 5.40$, $p = 0.07$, Fig. 2.4e) in the absence of barbels, whereas the ratio for predator:prey richness increased significantly (richness: $\chi^2 = 12.00$, $p = 0.002$; rarefied richness: $\chi^2 = 9.92$, $p = 0.007$; Fig. 2.4f).

Gut content analysis revealed that predatory invertebrates (secondary consumers) amounted to, on average, 22.8 ± 3.5% (mean ± SE) of the individuals in the barbels' gut contents. The most abundant predators found in the gut contents were *Zavrelimyia* sp. (a chironomid), *Parasigara* sp. (a hemipteran), and *Stictonectes* sp. (a water beetle). Other predatory invertebrates including Odonates (such as Lestidae, Libellulidae, and

Aeshnidae families) and other water beetles (such as *Agabus* sp. or *Nebrioporus* sp.) were also found in *B. meridionalis* gut contents (Table S.2).

Periphyton primary production declined in the absence of the top predator ($\chi^2 = 17.82$, $p < 0.001$; Fig. 2.5, Table S.1).

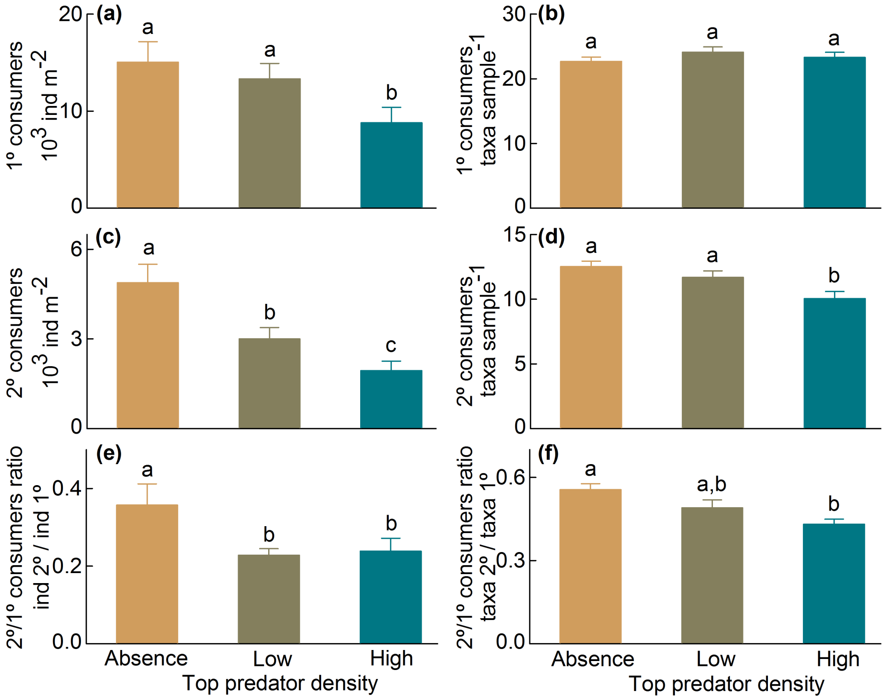


Figure 2.4 *Barbus meridionalis* density effects on macroinvertebrate abundance (mean ± SE individuals m^{-2}) and rarefied taxa richness (mean ± SE rarefied taxa $sample^{-1}$) for: 1º consumers (a-b), 2º consumers (c-d), and the ratio of 2º to 1º consumers (mean ± SE ratio $sample^{-1}$) (e-f). Different letters correspond to significant differences resulting from the pairwise comparisons among treatments (*U*-test, $p < 0.05$).

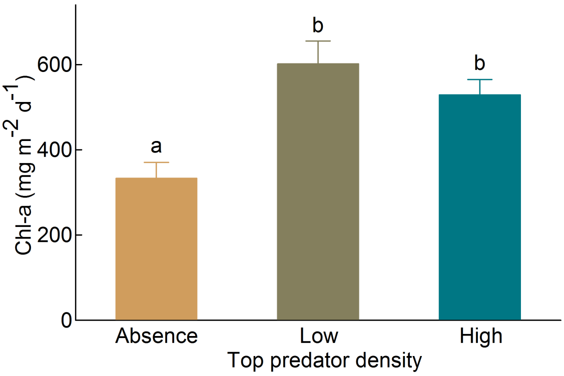


Figure 2.5 Periphyton net primary production measured as the chlorophyll-*a* on tiles for the three experimental treatments. Bars represent mean ± SE (mg m^{-2} d^{-1}). Different letters correspond to significant differences resulting from the pairwise comparisons among treatments (*U*-test, $p < 0.05$).

Discussion

This study demonstrated that apex consumer extinctions in intermittent streams may result in major changes to the system's structure and function. Like others (Ritchie & Johnson 2009; Prugh *et al.* 2009; Beschta & Ripple 2009; Brashares *et al.* 2010), our study showed how a top predator extirpation led to 'mesopredator release' in terms of abundance and richness. More importantly, top predator loss led to 'prey release', which contrasts with traditional food web theory and IGP literature. In addition, it triggered a trophic cascade that reduced periphyton primary production. Macroinvertebrate community composition also changed due to *B. meridionalis* absence. These results, along with other studies done in temporary salt marshes (Compte *et al.* 2012) and streams (Woodward *et al.* 2008; Gido *et al.* 2010), support that the effects of the loss of small-bodied fish are equivalent to local extinctions of larger apex consumers in other ecosystems (e.g. the arctic fox, wolf, jaguar, sea otter, or large reef fish (Beschta & Ripple 2009; Estes *et al.* 2011; Ripple *et al.* 2013)). Most studies about the consequences of the extinctions of top predators have been focused on large-bodied predators in terrestrial an marine systems (Ritchie & Johnson 2009; Prugh *et al.* 2009), usually associating large-bodied species to the top of the food webs and small-bodied species to lower trophic levels (Marshall & Essington 2011; Säterberg *et al.* 2013), positing also that large-bodied species are at much greater extinction risk than smaller species (see Cardillo 2003). However, as showed in this study, small-bodied fish in aquatic ecosystems may also exert strong top-down effects, supporting the idea that 'keystone species' is not a body-size dependent concept, but 'keystone species' are those species whose effects in the ecosystem are disproportionate to their abundance (Paine 1969; Kotliar *et al.* 1999). Moreover, Jenkins (2003) suggests that aquatic species, in particular freshwater fish, are more vulnerable to extinction than terrestrial species, and Olden *et al.* (2007) highlight that the most globally threatened freshwater fish are small-bodied species. Putting together the results of this study with the fact that numerous small-bodied freshwater fish are at extinction risk, it seems critical to persist in the consideration of the ecological consequences of their possible losses.

Ecosystem structure: 'mesopredator release' and 'prey release'

Mesopredators were more abundant in mesocosms lacking barbels, supporting the 'mesopredator release' hypothesis (see Fig. 2.1b), which confirms that the loss of small-bodied top predators may have this main common effect with large-bodied predator extirpations (Ritchie & Johnson 2009; Prugh *et al.* 2009; Beschta & Ripple 2009; Brashares *et al.* 2010). Several predatory invertebrates that characterized the enclosures lacking barbels (e.g. *Zavrelimyia* sp., *Parasigara* sp., and *Stictonectes* sp.; see Table 2.1) dominated barbel gut contents, indicating that fish predation contributed to density reduction for these taxa in the presence of barbels. Other taxa, such as *Chaoborus* sp., were not found in barbel gut contents, suggesting that the density decline for some taxa was most likely the result of induced emigration. Mesopredator abundance thus appears to be controlled by the top predator through the combination of predation and possible non-consumption impacts such as competition or induced emigration. Moreover, mesopredator richness also increased in top predator absence. Consequently, a basic element of trophic webs was altered (Elton 1927): predator:prey ratios differed among the barbel density treatments (see Fig. 2.4e-f). Even though predator:prey richness ratio has been previously considered invariant due to underlying community assembly rules (Cohen 1977; Warren & Gaston 1992; Jeffries 2002), our results support other studies that did not find conservative predator:prey ratios (Wilson 1996; Shulman & Chase 2007) and suggest that secondary and primary consumers respond unequally to the presence of a top predator.

'Mesopredator release' did not lead to a negative or a null effect on primary consumers (see Fig. 2.4a), which conflicts with the original IGP theory (Polis *et al.* 1989; Rosenheim *et al.* 1995; Snyder & Ives 2001; Finke & Denno 2005). In contrast, top predator absence led to increased primary consumer abundance (i.e. 'prey release'), which indicates that the top predator was more effective than mesopredators at suppressing prey. A growing body of literature has posited that top predator presence does not necessarily lead to higher prey abundance if the mesopredator exclusively uses alternate prey (Holt & Huxel 2007) or is cannibalistic (Rudolf 2007). However, these new perspectives on IGP are difficult to apply in empirical studies because models continue to oversimplify real food webs (e.g. by modelling food webs with just one intermediate predator). The IGP meta-analysis of Vance-Chalcraft *et al.* (2007) concluded that top predator presence usually leads to 'prey release', as predicted by trophic cascade theory. However, they also suggested that it is unclear in lotic ecosystems. In this sense, our results showed that the role of the apex consumer was not functionally replaced by the remaining species (Ernest & Brown 2001; Chalcraft & Resetarits 2003), suggesting that the predator assemblage is more important than diversity *per se* (Cardinale *et al.* 2006; Schneider & Brose 2013), with species identity being the critical factor.

Our study confirmed top predator extirpation modified the whole community composition. This finding was previously reported for intermittent streams exclusively by Williams *et al.* (2003), who found fish have a top-down effect on macroinvertebrate assemblages in isolated pools. But to our knowledge, our study is the first in demonstrating top predator extirpation can change community composition in a running intermittent stream. The treatment lacking barbels was the only that contained a large number of associated indicator taxa (see Table 2.1). Therefore, the presence of *B. meridionalis* prompted a macroinvertebrate community that was a subset of the macroinvertebrate community without the top predator. The responses of invertebrate populations to barbel presence were highly taxon-dependent, which supports evidence elsewhere that taxa within a trophic level are not functionally equivalent (Chalcraft & Resetarits 2003; Schmitz *et al.* 2004). No taxon was, however, positively affected by barbel presence. We found a statistically significant response even from highly mobile taxa that could rapidly recolonize the enclosures by drift (Gilliam, Fraser & Sabat 1989; Woodward *et al.* 2008), indicating a strong top-predator impact. These results indicate that some invertebrates have difficulty co-occurring with this apex consumer. Thus, the local extinction of *B. meridionalis* offered a competitive advantage for these vulnerable species to predation, and did not lead to an extinction cascade, which conflicts with the predator-mediated coexistence theory (Caswell 1978). Likewise, it contrasts with several studies that relate top predator extinctions to a decline in biodiversity (Henke & Bryant 1999; Ritchie & Johnson 2009); we did not find a relationship between top predator loss and total taxa richness or Simpson's diversity, only for mesopredator richness that increased in top predator absence.

Several studies have emphasized that top predators may be functionally extinct from an ecosystem before being extirpated (Soulé *et al.* 2003; Beschta & Ripple 2009; Säterberg *et al.* 2013). Management efforts to maintain threatened top predators at persistent levels can be ecologically irrelevant if the top predator population does not reach a functionally effective abundance. In our study, the top predator at low density (i.e. prefire density) led to an effective suppression of mesopredators, modified the whole macroinvertebrate community composition, and increased indirectly periphyton primary production compared to the treatment without barbels. However, part of the top predator functional role was only revealed at higher fish density, since the suppression of mesopredator richness and primary consumers' abundance did not occur at low top predator density. These results place apex consumer density as a continuum factor that modulates top predator effects in the ecosystem, confirming that studies about functional extinction thresholds that research top-down effects of apex consumers' extinctions at different densities are particularly relevant for ecosystem restoration and conservation purposes.

Ecosystem function: primary production response

Periphyton net primary production was significantly lower in the absence of *B. meridionalis* (see Fig. 2.5), confirming a strong trophic cascade effect that modified ecosystem function. This effect could occur through several different mechanisms, which are not necessarily mutually exclusive. Changes in primary consumer density could not fully explain the decline in primary production in top predator absence (see Fig. 2.4a). However, primary production could be top-down controlled by one or more taxa due to differences in the strength of this interaction, with herbivore identity being the key in the herbivore-producer interface. In this case, *B. meridionalis* extirpation could have increased the abundance of taxa that placed strong pressures on periphyton, triggering a trophic cascade without increasing the total abundance of primary consumers. Another explanation could be that predatory invertebrates were actually omnivorous, and 'mesopredator release' (see Fig. 2.4c) led to the increased consumption of periphyton. In addition to density-dependent causes, top predator presence could have led to higher primary production through a trait-mediated effect, reducing foraging activity by herbivores (Schmitz *et al.* 2004). Although positive interactions have been poorly studied by benthologists (Holomuzki *et al.* 2010), the presence of *B. meridionalis* could have had a direct positive effect on periphyton production via nutrient release and/or by increasing light levels as a result of reduced sediment deposition through feeding foraging movements (Ludlam & Magoulick 2010). These results demonstrate that trophic cascades can be strengthened at the herbivore-producer interface, and conflict with those of Shurin *et al.* (2002), which established that predators more strongly affected primary consumers compared to producers.

Our results regarding primary production have implications for the management of natural and human-altered ecosystems. For instance, our results could modify the general view of how predatory fish abundance is linked to primary production in freshwater ecosystems, given that our results conflicted with traditional trophic cascade theory (which holds that each trophic level is related to the level above and below it in a direct and negative way (Carpenter *et al.* 1985)). In agroecosystems, biological-control practitioners often consider IGP, a very common interaction among aphidophagous predators and parasitoids (Rosenheim *et al.* 1995; Gagnon, Heimpel & Brodeur 2011). In this context, Finke & Denno (2005) advised against promoting diverse predator assemblages in which IGP was common because it would weaken the suppression of herbivore pests and reduce productivity. These kinds of generalizations can lead to ineffective management practices, particularly given that our results showed that IGP did not dampen the trophic cascade and that neither IGP nor diversity were linked to cascade strength. Instead, and in agreement with Borer *et al.* (2005), cascade strength depended on the identity of predators and herbivores. Therefore, we recommend that managers place more importance on species identity in decision-making processes to better predict management outcomes.

Conclusions and implications

We conclude that intermittent streams may be affected by the consequences of top predator extinctions. In this study, the apex consumer was functionally irreplaceable despite its small-bodied size and even at low population densities. Indeed, the local extinction of *B. meridionalis* led to the loss of an important functional role that resulted in major changes to the ecosystem. Top predator absence triggered a 'mesopredator release', but also a 'prey release', and changed the whole macroinvertebrate community composition. Regarding ecosystem function, periphyton primary production declined indirectly due to top predator loss. We highlighted that the consequences of this species loss were unforeseen, particularly given that our results were not supported by traditional food web theory. Which ecological responses in mesocosms can be extrapolated to real ecosystems is an open ecological question (Lamberti & Steinman 1993). Brown *et al.* (2011) demonstrated that aquatic mesocosms can reproduce replicable and realistically not just physicochemistry and macroinvertebrate community composition, but complex food webs. Our in-stream mesocosms were carefully design to not be a methodological artefact: mesh size allowed macroinvertebrate emigration/inmigration and complex tray substrates within the mesocosms provided refuge to macroinvertebrates. However, spatial complexity and refuge diversity were probably lower in the mesocosms compared to natural stream conditions, which may have increased predator-prey encounter rates. On the other hand, we used conservative top predator densities (i.e. the stream's prefire average density and its double); however, *B. meridionalis* can reach higher densities in stream isolated pools during the dry period (up to 20 ind m^{-2}, usually in summer) suggesting that the impact of this top predator could be even higher than observed here. Thus, despite of the limitations of our study, our main result is consistent: the extirpation of a small-bodied top predator can lead to deep system changes in intermittent streams, at least in the hydrological conditions during our experiment. However, research at larger spatial and temporal scales is needed to integrate the impact of hydrological variability in intermittent streams.

Small-bodied freshwater fish species usually lack commercial value and are often overlooked in conservation management even when considered threatened (Williams 2006; Saddlier *et al.* 2013). Based on our results, we recommend that reintroduction programs be considered for small-bodied fish in intermittent streams, where species such *B. meridionalis* had become extirpated. Reintroduction programs would allow not just for recovery of endangered species populations (e.g. *B. meridionalis*), but for the restoration of the ecosystem. Likewise, reintroductions should be considered within a restoration ecology framework, not focusing on mere species presence, but on ecological effectiveness. Because habitat fragmentation often drives apex consumer extirpations (Brashares *et al.* 2010; Staddon *et al.* 2010) and can hinder following natural recolonization, we also recommend the improvement of ecosystem connectivity as a preventive tool as well as a first step in restoration programs. In the context of freshwater ecosystems' conservation, given the high extinction risk of small-bodied freshwater fish, our study evidences that unpredictable ecosystem changes in these ecosystems may occur if conservation efforts are not undertaken.

Chapter 3

A trait-based approach reveals the feeding selectivity of a small endangered Mediterranean fish

Functional traits are growing in popularity in modern ecology, but feeding studies remain firmly rooted in a taxonomic-based perspective. Due to its assemblage specificity, the taxon-based approach limits our ability to develop and test a priori hypotheses across systems. Moreover, consumers do not have any reason to select their prey using a taxonomic criterion. We propose a simple method with a functional perspective to study the feeding selectivity of predators, based on the hypothesis that predators select their prey depending on their morphological and behavioral traits. We apply this trait-based approach to study prey selection by the endangered fish *Barbus meridionalis* in a Mediterranean stream. Feeding selectivity was inferred by comparing the traits and taxonomic composition of the ingested prey and free-living potential prey using the Jacob's electivity index. We analyzed 13 prey traits with 55 trait categories. The diet of *B. meridionalis* was dominated by chironomids and ephemeropterans. Fish refused most of the potential prey in the stream but positively selected *Cricotopus* spp., *Habrophlebia* sp., and *Stictonectes* sp. The trait-based analysis showed that 10 of the 13 traits tested significantly influenced food choice: body size, body shape, body flexibility, concealment, locomotion, tendency to drift, diel drift behavior, agility, aggregation tendency, and feeding habits. Our study shows that morphological and behavioral traits may explain prey vulnerability to predation. This trait-based approach is a promising perspective to improve our understanding of predator-prey interactions, to make cross-ecosystem comparisons through changing species-assemblages and, consequently, to predict the ecosystem impacts of predator invasions and extinctions.

Introduction

Studies on fish feeding ecology are central to understanding trophic, material, and energy dynamics, to model precise outcomes for each system, and to develop conservation strategies for species and ecosystems (Braga *et al.* 2012). Current biodiversity loss is biased towards species in the higher trophic levels (Duffy 2002; Schneider & Brose 2013), freshwater fish being among the most threatened fauna worldwide, particularly the small-bodied ones (Jenkins 2003; Olden *et al.* 2007). Besides having an inherent conservation value, the loss of these species can trigger major changes in ecosystems because they often act as top-predators such as occurred in intermittent streams (Rodríguez-Lozano *et al.* 2015b). However, our understanding of how these endangered species influence food-webs remains limited, mainly because most of the feeding ecology studies in freshwater fish have been focused on commercial species (Braga *et al.* 2012).

Traditionally, studies on selective predation have used a taxon-based approach, even though consumers do not have any reason to select their prey using a taxonomic criterion. According to the optimal foraging theory (OFT), predators select their prey in order to maximize their net rate of energy gain in relation to the energetic costs (Pyke 1984). In this trade-off, prey size has been claimed to be the primary determinant of predator choice because it reflects well the costs (e.g. handling time) and benefits of foraging (e.g. prey energy content) (Werner & Hall 1974; Woodward & Warren 2007). In fact, recent food-web modelling includes the predator-prey allometric relationship to better predict the structure of food webs (Petchey *et al.* 2008; Klecka 2014). However, other traits, either morphological (e.g. concealment and body shape) or behavioral (e.g. drift tendency and prey movements), may also determine the predator optimal foraging strategy (Rader 1997; de Crespin de Billy & Usseglio-Polatera 2002; Allan & Castillo 2007; Klecka & Boukal 2013). Therefore, the exploration of the role of prey morphological or behavioral traits other than body size in predator choice may help increase the accuracy and ecological realism of food-web studies (Ings *et al.* 2009; Rohr *et al.* 2010; Boukal 2014; Klecka 2014).

A trait-based approach has been further applied in a descriptive way to study fish diet (e.g. de Crespin de Billy and Usseglio-Polatera 2002, Sánchez-Hernández *et al.* 2012). However, only one recent study by Green and Côté (2014) has examined the selectivity of a fish predator on prey traits. This functional perspective presents a framework to better understand predator-prey interactions. Furthermore, since macroinvertebrate assemblages are variable in space and time, the use of prey traits is likely to facilitate cross-study comparisons (Green & Côté 2014). In this regard, if the preferred prey traits of a fish species are known, it may help predict their effects on ecosystems. Therefore, studies on predator selectivity on prey traits could better predict the ecological consequences of native species extirpation and invasive species introduction, thus improving our understanding of how anthropogenic impacts may influence food web structure and dynamics.

The present study explored the benefits of using a common selectivity index in a trait-based perspective, through the study of feeding ecology of the endangered freshwater fish *Barbus meridionalis* (A. Risso, 1827). This species is endemic to NE Spain and SE France, and is currently listed as 'vulnerable' in the Spanish Red Book (Doadrio 2001), 'near threatened' in the IUCN and is also included in Annexes II and V of the European Union Habitats Directive and in Appendix III ('protected fauna species') of the Bern Convention. Similar to other freshwater fish species, the list of threats include water pollution, water abstraction, dam construction, introduced species, and alterations in habitat (Doadrio *et al.* 2011; Maceda-Veiga 2013). Despite the scarce information on *B. meridionalis* feeding ecology, this species has been considered a benthic invertivore species (Doadrio *et al.* 2011) that feeds primarily on chironomid larvae, detritus, mayflies, amphipods, isopods, and terrestrial invertebrates (García-Berthou 1994; Mas-Martí *et al.* 2010). Thus, *B. meridionalis* fits well as a case-study to explore the utility of multi-trait approaches in examining the selective predation of a top consumer.

Materials and methods

Study area

The study was carried out in the Vall d'Horta stream within the protected area of Sant Llorenç del Munt i l'Obac Natural Park (50 km inland from Barcelona city, NE Spain). This area has calcareous geology and is under the Mediterranean-climate domain, with mild winter and warm spring and summer. This area is dominated by Holm oak (*Quercus ilex* L.) and Aleppo pine (*Pinus halepensis* Miller) forests and Mediterranean shrubs (see Bonada *et al.* 2007 for a detailed site description). In intermittent streams, such as the Vall d'Horta, *B. meridionalis* can be the major top-down control of the aquatic community (Rodríguez-Lozano *et al.* 2015b). This species uses permanent pools as refuges during periods of hydrological disconnection (usually in summer), as do other fish species in this region (Aparicio & de Sostoa 1999). In August 2003, a wildfire burned a forested area of 4543 ha and, consequently, *B. meridionalis* was locally extinct in some streams, most likely due to water quality deterioration (Vila-Escalé *et al.* 2007a). The fish population has not recovered since then, possibly due to the presence of natural and human barriers downstream.

Field and laboratory work

In this study, we used the benthic macroinvertebrates and gut content data from a previous study, which also provides full details on the experimental design (Rodríguez-Lozano *et al.* 2015b). We carried out an enclosure experiment in the Vall d'Horta stream (41°40'24''N, 2°02'4''E; Altitude: 480 m a.s.l.), a first order stream in the Besòs river basin. The experiment was run for five weeks in late spring in 2010 before pool disconnection (flow averaged 15.7 ± 0.9 L s^{-1}). The mesocosm experiment consisted of nine large cages (100 x 100 cm surface, 70 cm height) of 10-mm mesh size, enabling macroinvertebrates to pass through. We randomly assigned three barbel densities to cages (3 cages per treatment) in order to simulate: fish extirpation, known prefire fish density (2 individuals m^{-2}, A. de Sostoa pers. comm.), and a two fold increase in prefire density (4 individuals m^{-2}). Each cage contained four trays (with stones and glass tiles) that were left for three weeks in order to allow for establishment of the macroinvertebrate community (for further description see Rodríguez-Lozano *et al.* 2015).

Fish were caught using electrofishing downstream from our study site. Eighteen individuals were size-matched (total length: 101.8 ± 2.6 mm; weight: 2.3 ± 0.2 g mean ± SE) and kept for observation, caged in the stream, for 24 h prior to the experiment. After two weeks in the mesocosms, fish individuals were euthanized using an overdose of the anesthetic MS-222® (Tricaine methane-sulfonate, Sigma-Aldrich), measured (total length, ± 1 mm), weighed (± 0.01 g), dissected, and the entire guts were preserved in 4% formalin. To quantify the potential prey, the content of each tray was carefully sieved through a 250-µm mesh and individually preserved in 4% formalin.

In the laboratory, we sorted and counted all macroinvertebrates in gut and benthos samples under the stereomicroscope. All taxa were identified to the genus level, with the exception of some dipterans (family level) and Oligochaeta, Ostracoda, Cladocera, Copepoda, Hydracarina, and terrestrial invertebrates. The same taxonomic resolution was used for free-living and ingested prey.

Data analysis

For the taxonomic approach, we estimated the relative importance of each taxon by determining the relative abundance of each prey item (i.e. the number of individuals of a prey in a gut divided by the total number of individuals, in percentage) and their frequency of occurrence (i.e. the percentage of guts in which a prey was present). To visualize the prey importance and feeding strategy we used the graphical Costello method. The plot shows the relative abundance of prey vs their frequency of occurrence, meaning that points in the top left corner indicate a diet specialization of some individuals (Costello, Edwards & Potts 1990). Diet diversity was calculated using the Shannon-Wiener index ($H'=-\sum P_i \log_{10} P_i$, where P_i is the proportion of the diet that is represented by prey item *i*). Moreover, the specialization in the diet evaluated using Pielou's evenness index ($J=H'/H'_{max}$). We considered that J values close to zero indicate a stenophagous diet, while J values close to one indicate a euryphagous diet (Oscoz *et al.* 2005).

To analyze the taxonomical feeding selectivity of *B. meridionalis*, we compared macroinvertebrates found in the gut contents with the macroinvertebrate community. Feeding selectivity was measured using Jacob's index of electivity *D* (Jacobs 1974), calculated as D = r−p/(r + p−2rp), where r is the proportion of the diet accounted for by a given prey taxon, and p is the proportion of the taxon per predator cage accounted for by that taxon. D varies from −1 to 0 for negative selection, and from 0 to +1 for positive selection. To test whether selectivity significantly deviated from 0, a one-sample nonparametric test (Wilcoxon Signed Rank test) was used, as data were not normally distributed. Since chironomids are rarely identified to the genus level, we explored the importance of taxonomic resolution on the measure of feeding selectivity by comparing outputs for chironomids at the family, subfamily, and genus level. This family was also selected because from our experience Chironomidae is the most diverse family in these streams. Moreover, we explored the importance of fish density in diet diversity and feeding selectivity by using an ANCOVA test with fish density as fixed factor and fish length as covariable.

For the trait-based approach, we collected 13 macroinvertebrate traits with 55 trait categories (see Table 3.1) from public depositories (de Crespin de Billy 2001; Tachet *et al.* 2010). We used five morphological traits related to handling efficiency (invertebrate 'potential size', 'body shape', 'body flexibility', 'concealment' ability, and potential 'morphological defenses' such as cerci or spines) and eight behavioral traits ('locomotion and substrate relation', 'tendency to drift in the water column', 'diel drift behavior', 'agility', 'movement frequency', 'trajectory' trait, 'aggregation tendency', and 'feeding habits'). A score between 0 and 5 was assigned to each taxon for each trait using a fuzzy coding approach (Chevenet, Dolédec & Chessel 1994), with '0' indicating 'no affinity', and '5' indicating 'high affinity'. Given that some genera (mostly Chironomidae) were not included in public trait databases, these were coded using: other published information (e.g. Puntí, Rieradevall & Prat 2009), the available information at subfamily or family level, the mean of other genera values within the same family, and the personal experience of the senior authors of this paper. Ostracoda, Cladocera, Copepoda, Hidracarina, tadpoles, and terrestrial invertebrates were not included in the trait analysis due to the lack of trait information.

Table 3.1 Traits, categories, and codes used in analyses and graphics.

Traits	Categories	Codes
Potential size (mm)	≤ 2.5	<2.5
	2.5 - 5	2.5-5
	5 - 10	5-10
	10 - 20	10-20
	20 - 40	20-40
	40 - 80	40-80
	> 80	>80
Body shape (including cases/tubes)	Cylindrical	cyl
	Spherical	sph
	Conical	con
	Flattened	flat
Body flexibility (including cases/tubes)	None	f.no
	Weak	f.weak
	High	f.high
Concealment	Fixed accessory	net
	Movable accessory	case
	Solidly colored	c.sol
	Variable	c.var
	Patterned	c.patt
Morphological defenses	Cerci, silk, spine	def.sp
	None	def.no
Locomotion and substrate relation	Surface swimmer	surf.s
	Full water swimmer	wat.s
	Crawler	craw
	Burrower	burw
	Interstitial	int
	Attached	att
Tendency to drift in the water column	None	d.no
	Weak	d.weak
	Medium	d.med
	High	d.high
Diel drift behavior	None	none
	Nocturnal	noct
	Dawn	dawn
	Daylight	d.light
	Twilight	t.light
Agility	None	a.no
	Weak	a.weak
	High	a.high
Movement frequency	Continuous	cont
	Discontinuous	disc
Trajectory on the bottom substratum or in the drift	None	t.no
	Linear	t.lin
	By random	t.rand
	Oscillatory	t.oscil
Aggregation tendency	Weak	ag.weak
	High	ag.high
Feeding habits	Absorber	abs
	Deposit	dpfd
	Shredder	shrd
	Scraper	scrp
	Filter-feeder	filt
	Piercer	pier
	Predator	pred
	Parasite	par

We inferred the selection of *B. meridionalis* for prey traits as we did for the taxonomical feeding selectivity. We compared the traits of the macroinvertebrates found in the gut contents with the traits of the benthic macroinvertebrate community. For each trait category, Jacob' selectivity index was calculated and Wilcoxon Signed Rank test was computed to test whether selectivity was statistically significant. All statistical analyses were performed in R 2.15.2. (R Core Team 2012).

Ethical note

This study was approved by the Autonomous Government of Catalonia (Generalitat de Catalunya) and the Natural Parks Department of the Government of Barcelona (Diputació de Barcelona). This authorization only enabled us to sacrifice 18 fish that were euthanized following the procedure used in the aquatic animal facility at the University of Barcelona. All efforts were made to minimize animal stress, and individuals captured represented 10% of the fish in the donor population.

Results

Taxon-based diet analysis

Overall, 38 different taxa were found in *B. meridionalis* guts. Mean prey abundance in gut contents was 161 ± 30 (mean ± SE) individuals. *Barbus meridionalis*' diet was dominated by chironomids (71.3 ± 5.0%) and ephemeropterans (15.5 ± 2.2%), with *Cricotopus* spp. and *Habrophlebia* sp. present in all guts (Fig. 3.1, Table S.2). Other taxonomical groups were also frequently found, such as coleopteran larvae (e.g. *Stictonectes* sp., *Agabus* sp.), heteropterans (e.g. *Parasigara* sp.), gastropods (e.g. *Gyraulus* sp., *Physella* sp.), and odonates (e.g. *Chalcolestes viridis*, *Sympetrum* sp.). In contrast, caddisflies (e.g. *Tinodes* sp.), small crustaceans, leeches, and terrestrial invertebrates were rare. Dietary descriptors showed that prey abundance predominated over occurrence in describing fish diet (Fig. 3.1), suggesting that all fish prey on similar items. Diet diversity (0.62 ± 0.04) and the evenness index (0.68 ±

0.05) further supported that these fish were euryphagous. We did not quantify the contribution of detritus to fish diet, but it was barely present in all gut contents.

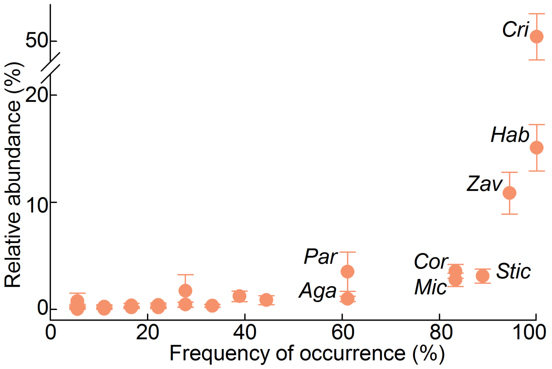


Figure 3.1 Relationship between relative abundance (%) and frequency of occurrence (%) of food categories in the *Barbus meridionalis* gut contents based on Costello's method. The written food categories represent the most important prey items. Cri-*Cricotopus* spp., Hab-*Habrophlebia* sp., Zav-*Zavrelimyia* sp., Stic-*Stictonectes* sp., Cor-*Corynoneura* spp., Mic-*Microtendipes* sp., Par-*Parasigara* sp., Aga-*Agabus* sp.

The prey electivity index revealed that *B. meridionalis* fed on particular prey, ignoring many potential prey (D = -0.70 ± 0.02; $p < 0.001$). Surprisingly, these discarded taxa included some of the most abundant chironomids, such as *Tanytarsus* sp. and *Dicrotendipes* sp. (Figs 3.2, 3.3). In contrast, other abundant taxa, such as *Cricotopus* spp. (D = 0.77 ± 0.05; $p < 0.001$), *Habrophlebia* sp. (D = 0.44 ± 0.10; $p < 0.001$), and *Stictonectes* sp. (D = 0.57 ± 0.14; $p < 0.03$) were highly positively selected by *B. meridionalis* (Fig. 3.2). Proportionally, *B. meridionalis* ate less chironomids than were in the benthos, with the exception of *Cricotopus* spp. (Fig. 3.3). However, this selective predation of *B. meridionalis* on chironomids was only observed at the subfamily and the genus levels, but not at the family level (D = -0.03 ± 0.11; $p = 0.77$; Fig. 3.3).

Fish density had no effect on diet diversity or on mean prey selectivity. However, diet diversity increased with fish length ($F = 17.87$, $p < 0.001$), and larger fish individuals discarded less taxa ($F = 12.22$, $p < 0.003$).

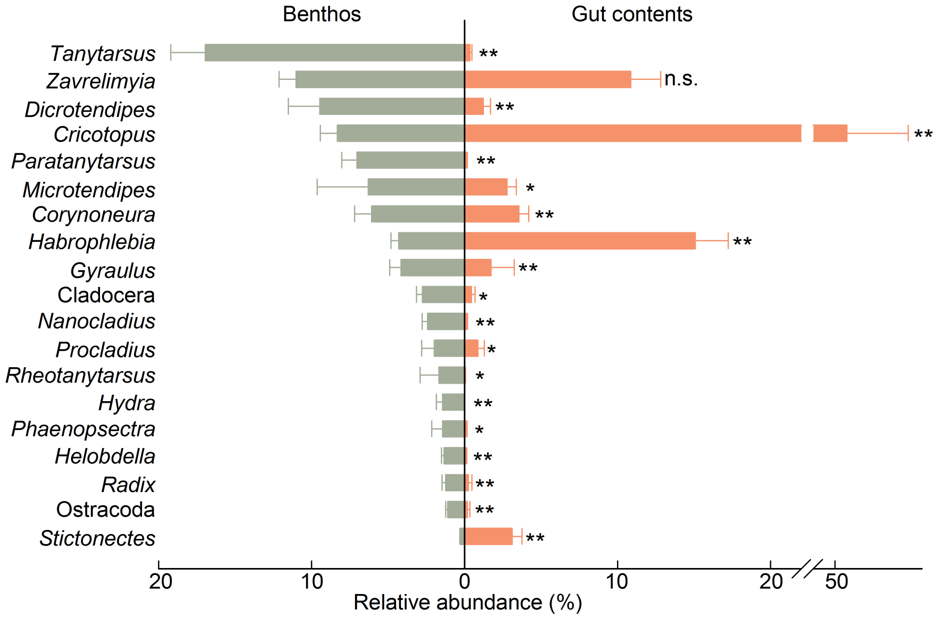


Figure 3.2 Relative abundance of the mesocosm taxa compared to its relative abundance in gut contents. Taxa are ordered by their abundance in the mesocosm benthos and only the most abundant ones (>1%) are presented. The statistically significant thresholds are: *$p < 0.05$, **$p < 0.01$.

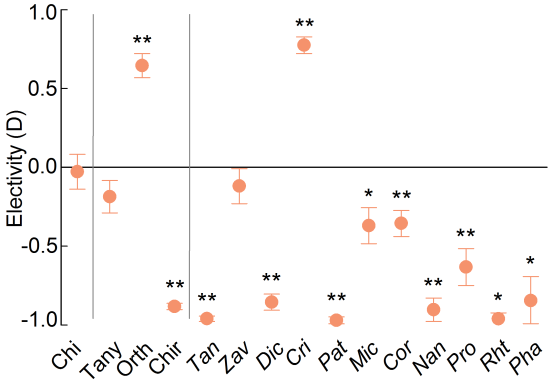


Figure 3.3 Selectivity of *Barbus meridionalis* for the Chironomidae family, subfamilies, and genera. Chi-Chironomidae family; Tany-Tanypodinae subfamily, Orth-Orthocladinae subfamily, Chir-Chironominae subfamily; Tan-*Tanytarsus* sp., Zav-*Zavrelimyia* sp., Dic-*Dicrotendipes* sp., Cri-*Cricotopus* spp., Pat-*Paratanytarsus* sp., Mic-*Microtendipes* sp., Cor-*Corynoneura* spp., Nan-*Nanocladius* sp., Pro-*Procladius* sp., Rht-*Rheotanytarsus* sp., Pha-*Phaenopseptra* sp. The different genera are ordered by their abundance found in the mesocosms. The statistically significant thresholds are: *$p < 0.05$, **$p < 0.01$.

Trait-based diet analysis

The selective predation of *B. meridionalis* for particular taxa was mirrored in the trait approach. *Barbus meridionalis* significantly selected 60% of the trait categories (i.e. 33 of the 55 trait categories). Refuse choices were more common than positive ones: 24 trait categories were negatively selected, while 9 trait categories were positively selected (Fig. 3.4). Specifically, fish fed mostly on macroinvertebrates with a potential size of 5-10 mm (72.5 ± 1.3%), with a cylindrical body shape (87.8 ± 3.2%), and a high body flexibility (77.6 ± 4.3%). In contrast, fish seemed to avoid small and big potential prey, prey with conical shape (D = -0.83 ± 0.12, $p < 0.001$), and prey without body flexibility (D = -0.47 ± 0.14, $p = 0.006$). Macroinvertebrate concealment ability also had a significant effect on barbel selectivity. Fish fed mostly on solidly colored prey (58.2 ± 1.8%) and selected positively variable colored prey (D = 0.53 ± 0.04, $p < 0.001$). However, prey with patterned color or with fixed or movable accessories (i.e. nets, retreats, cases, or tubes) were less vulnerable to fish predation (Fig. 3.4). Most prey did not have morphological defenses (88.8 ± 1.6%), but *B. meridionalis* did not significantly select this trait category.

Regarding macroinvertebrate locomotion, crawlers were the most abundant in gut contents (38.0 ± 2.4%) followed by attached macroinvertebrates, burrowers, and full water swimmers. However, fish negatively selected surface swimmers (D = -0.78 ± 0.10, $p < 0.001$) and interstitial macroinvertebrates (D = -0.56 ± 0.06, $p < 0.001$). Interestingly, prey with high aggregation tendency dominated gut contents (70.7 ± 0.8%), but fish preferred prey with weak aggregation tendency (D = 0.13 ± 0.02, $p < 0.001$). Similarly, barbels positively selected prey with medium and high drift tendency (medium drift: D = 0.09 ± 0.02, $p = 0.003$; high drift: D = 0.40 ± 0.05, $p < 0.001$), even though those with weak drift tendency predominated in fish guts (52.1 ± 2.2%). In particular, macroinvertebrates that drift during daylight were positively selected (D = 0.16 ± 0.06, $p = 0.030$). Most prey eaten by *B. meridionalis* also had a discontinuous movement (58.1 ± 3.3%) with an oscillatory trajectory (59.0 ± 3.2%), but these traits (movement frequency and trajectory) were not retained as significant.

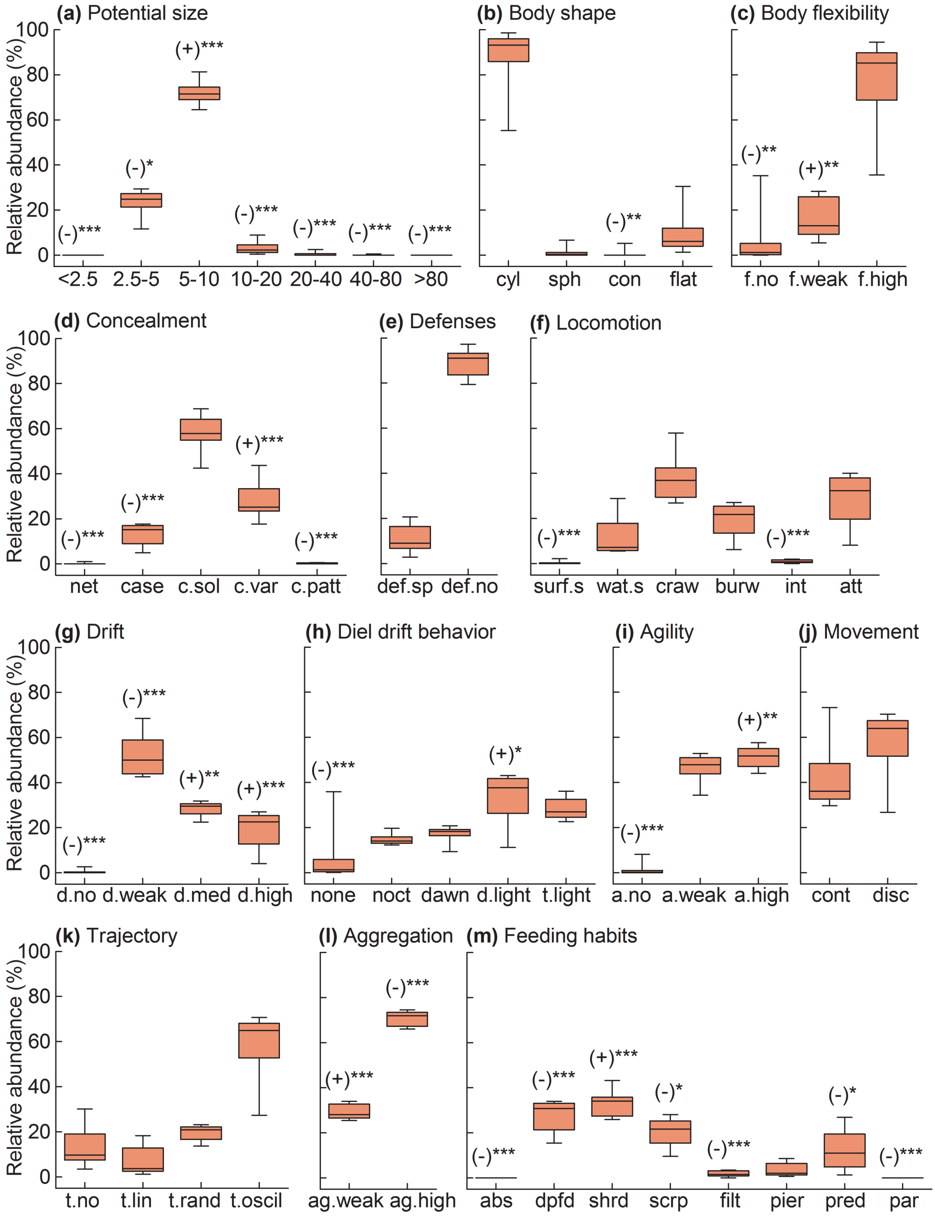


Figure 3.4 Relative importance of the 55 categories within the 13 studied traits in the *Barbus meridionalis* gut contents (for codes, see Table 3.1). Significant selectivity of trait categories is marked as: (+) positive, (-) negative, or no response. The statistically significant thresholds are: * $p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

In contrast, prey was selected by *B. meridionalis* in relation to their swimming or crawling speed, being highly agile macroinvertebrates positively selected (D = 0.15 ± 0.03, $p < 0.001$) and more often captured (51.1 ± 1.5%) than slow-moving prey (D = -0.80 ± 0.09, $p < 0.001$). According to trophic guilds, shredders were the most abundant feeding group in gut contents (33.2 ± 1.3%), followed by deposit feeders (27.6 ± 1.7%), scrapers (20.91 ± 1.85%), and predators (12.7 ± 2.2%). However, *B. meridionalis* only selected positively macroinvertebrate shredders (D = 0.63 ± 0.02, $p < 0.001$).

Discussion

Our study shows that taxonomic differences in the diet of *B. meridionalis* can be explained by morphological and behavioral traits. Several studies have used trait approaches to describe fish diet (e.g. de Crespin de Billy & Usseglio-Polatera 2002; Sánchez-Hernández *et al.* 2012) and to measure food choice based on prey size (e.g. Rincón & Lobón-Cerviá 1999; Sánchez-Hernández & Cobo 2015). However, only one recent study has examined predator selectivity on several prey traits (Green and Côté, 2014), for which the authors used linear mixed-effects models. Here, we propose the Jacob's D electivity index, widely used in food-choice studies (e.g. Copp, Spathari & Turmel 2005; Winkelmann *et al.* 2007; Lee & Suen 2014), as a simple tool to examine predator selectivity on prey traits. The benefits of using a trait-approach are evident in our study. For instance, the fact that more than 88% of ingested macroinvertebrates had no morphological defenses (i.e. cerci, silk, and spines) could be interpreted as fish avoidance of prey with morphological defenses. However, our analysis did not show a negative selection for this trait, suggesting that prey vulnerability to predation by *B. meridionalis* was not related to the presence of these morphological defenses.

Taxon-based diet analysis

Our results were consistent with previous studies showing that *B. meridionalis* mostly feed on the larvae of chironomids (*Cricotopus* spp. and *Zavrelimyia* sp.) and mayflies (*Habrophlebia* sp.), followed by coleoptera larvae (*Stictonectes* sp. and *Agabus* sp.) and hemipterans (*Parasigara* sp.) (García-Berthou 1994; Mas-Martí *et al.* 2010). Similarly, other invertebrates besides insects were also found in the guts examined in our study (e.g. gastropods), supporting that *B. meridionalis* is invertivorous (Doadrio *et al.* 2011). While detritus was not quantified, its presence in fish guts seemed less predominant than in previous studies (Mas-Martí *et al.* 2010). These results are most likely attributed to the fact that fish ingested detritus unintentionally, at the same time that prey on benthic invertebrates. Conversely, it seems unlikely that fish ate detritus as a competitive adaptation, especially considering the high abundance of potential prey as well as the absence of interspecific competitors (Magalhaes 1992).

In the current study, the selectivity of *B. meridionalis* for macroinvertebrate taxa was both positive and negative. However, mean prey selectivity was predominantly negative as reported in previous studies (Mas-Martí *et al.* 2010). Our results show that several morphological and behavioral traits may explain predator food choice. Further explanations may include low energetic value and/or low palatability of some taxa (Sánchez-Hernández *et al.* 2011). Last but not least, the taxonomic resolution can influence our ability to infer fish feeding preferences, such as shown here with the Chironomidae family example. *Barbus meridionalis* selectivity for chironomids at subfamily and genus levels was obscured when the family level was used. Since chironomid taxa differ in microhabitat use, fish predation is most likely to vary accordingly. For example, *Cricotopus* spp. usually live on top of stones and are, therefore, more exposed to *B. meridionalis* predation than other chironomids that inhabit underneath the stones, such as *Tanytarsus* sp. and *Dicrotendipes* sp. Thus, taxonomic resolution may be a key factor in food-choice studies, as reported for food-web metrics (Thompson & Townsend 2000).

Contrary to the expectation of the optimal foraging theory (OFT), changes in diet breadth did not occur in *B. meridionalis* individuals between the density treatments. With high intraspecific competition, it was predicted that fish ingested a wider range of prey than at low density because it has been previously shown that fish feed on suboptimal prey when the preferred prey become scarce (Werner & Hall 1974; Martinussen, Robertsen & Einum 2011).

Our results suggested low intraspecific competition even when using high fish densities, at least for our experimental conditions. Nevertheless, higher competition is most likely to occur if *B. meridionalis* density reaches up to 20 ind m^{-2}; a condition that may happen during drought periods in isolated pools.

Trait prey selectivity

We provide new insights into prey-consumer interactions suggesting that up to 33 trait categories of 55 tested influenced prey choice. Most of these traits (e.g. the absence of body flexibility and/or the presence of nets or cases) were negatively selected by *B. meridionalis*. These results are consistent with the OFT theory (Pyke 1984), as prey with weak and high body flexibility are easier to handle. Similarly, prey with nets, cases, or with colored patterns seemed to reduce *B. meridionalis* predation by increasing prey concealment. Our results also indicated that surface swimmers and interstitial taxa escaped from fish predation when compared to crawlers, burrowers, and attached organisms, which could strengthen the benthic feeding behavior of *B. meridionalis* (Doadrio *et al.* 2011). However, pelagic taxa (i.e. full water swimmers) also occurred in the guts examined in this study, and were not negatively selected by *B. meridionalis*, indicating that *B. meridionalis* is benthopelagic.

Prey with a potential size of 5-10 mm were highly selected by *B. meridionalis*, whereas those larger and smaller than this size were avoided. Larger prey were negatively selected most likely because fish were gape-limited. These results highlight the importance of size refuge for prey (Chase 1999; Woodward *et al.* 2010), and suggest that body size could act as a bottleneck in diet choice. Although our study did not directly examined the effect of fish size on its diet, we found that large individuals of *B. meridionalis* fed on a wider variety of prey than the small ones and were less selective, thus contrasting with a previous study (Mas-Martí *et al.* 2010). The dependence of diet breadth and mean prey selectivity on fish length was most likely caused by the incorporation of larger prey into the diet of large fish (Woodward & Warren 2007).

Interestingly, contrasting patterns were found regarding *B. meridionalis* selectivity for macroinvertebrates according to their aggregation behavior. While taxa with high aggregation tendency predominated in the guts examined (>70%), the electivity index showed that *B. meridionalis* preferred those with weak aggregation tendency. Although aggregate assemblages make the group more conspicuous to predators, aggregation may be an anti-predator adaptation to dilute the predation impact among neighbors (Wrona & Dixon 1991) and to respond faster to detecting danger (Johannesen, Dunn & Morrell 2014). In addition to explaining prey vulnerability to predation *per se*, our study provides useful insights into how the use of prey traits may help us to better understand the role of fish in food-webs. Since shredders have an outstanding role in the litter decomposition process, our results suggest that the loss of top-down control by *B. meridionalis* may favor shredder activity, and hence, accelerate litter decomposition (Konishi, Nakano & Iwata 2001; Boyero, Rincón & Pearson 2008).

Conclusions

Our study suggests that the adoption of a trait-based perspective in studies on fish diet can improve our mechanistic understanding of prey-consumer relationships. Although body size was already reported as an important determinant factor for food choice, our study suggests that up to 33 trait categories within 10 traits may be involved in the feeding preferences of *B. meridionalis*. Our results also depicted some discrepancies with the existing literature in the habitat use and diet of this fish species, highlighting the need for more basic research into the biology and ecology of species. This also applies to macroinvertebrates because we need more basic knowledge to build more comprehensive public trait depositories (e.g. Vieira *et al.* 2006, Bonada and Dolédec 2011, Sánchez-Hernández *et al.* 2011). Finally, our study suggests that the trait-based perspective overcomes the limitations of the taxon- and size-based approaches, improving our knowledge of predator-prey interactions, facilitating cross-study comparisons and, consequently, helping to predict the outcomes of predator invasions and extinctions.

Chapter 4

Top predator absence enhances leaf breakdown in a Mediterranean stream

Current biodiversity loss is biased towards species in the higher trophic levels, small-bodied freshwater fish being among the most threatened fauna worldwide. In research studies and conservation management, small-bodied freshwater fish have often been overlooked because they usually lack commercial value. Therefore, the ecosystem impacts of their possible loss remain mostly unknown. In this study, we assessed the top-down impacts of an endangered small-bodied fish, *Barbus meridionalis* (A. Risso, 1827), in a detritus-based stream. We tried to determine whether the loss of a top predator affects leaf fungal biomass and leaf quality (i.e. leaf carbon:nitrogen ratio and leaf toughness), macroinvertebrate assemblages colonizing leaf packs and, consequently, leaf breakdown rates. In order to achieve these objectives, we conducted a leaf bag experiment in two adjacent reaches of an intermittent Mediterranean stream: a control downstream reach with a population of *B. meridionalis* (control site) and an upstream fishless reach. Top predator absence led to faster overall leaf decomposition, which was caused by the higher shredder and scraper biomass in coarse-mesh leaf bags in the fishless compared to the control reach. Fish absence reduced leaf fungal biomass in both fine- and coarse-mesh bags, but did not decrease microbially mediated leaf breakdown. These results suggest that leaf fungal biomass was stimulated from the bottom-up through nutrient recycling by the top predator. To our knowledge, the present study is the first to assess the impact of a predatory fish in leaf fungal biomass in a stream, as well as to explore the importance of fish on leaf decomposition in an intermittent stream.

Introduction

Our planet is experiencing the Sixth Mass Extinction, which is characterized by the loss of apex consumers (Duffy 2002; Estes *et al.* 2011; Schneider & Brose 2013). Top predators are acknowledged to exert a top-down force on their prey that can spread through the trophic web, leading to strong indirect effects (i.e. trophic cascades) (Hairston *et al.* 1960; Polis *et al.* 2000; Terborgh & Estes 2010). Trophic cascades have been documented in terrestrial, freshwater, and marine ecosystems, from the poles to the tropics, and most have focused on grazing systems (see Estes *et al.* 2011). In brief, ecosystem loss of apex consumers may alter the intensity of herbivory and, consequently, the abundance and/or composition of primary producers. However, our knowledge of the role of top predators in ecological networks is still limited, with an increasing body of literature highlighting that top-predator loss may trigger ecosystem changes that are complex, unpredictable, and largely unknown (Marshall & Essington 2011; Estes *et al.* 2011). Consequently, a current major challenge is to better understand the responses of ecosystems to the top-predator decline (Sutherland *et al.* 2013).

Most research regarding the consequences of the extinctions of top predators have been focused on large-bodied terrestrial mammals (Prugh *et al.* 2009; Beschta & Ripple 2009; Ordiz *et al.* 2013; Ripple *et al.* 2014), and secondarily on large marine predators (Heithaus *et al.* 2008; Ritchie & Johnson 2009), thus overlooking freshwater ecosystems. Moreover, most studies usually associate large-bodied species to the top of the food webs and small-bodied species to lower trophic levels (Marshall & Essington 2011; Säterberg *et al.* 2013). However, aquatic species seem more vulnerable to extinction than terrestrial species, freshwater fish being among the most threatened fauna worldwide (Jenkins 2003). In marine ecosystems larger fish are the most endangered, but in freshwater ecosystems small-bodied fish exhibit greater risk of extinction than their larger-bodied counterparts (Olden *et al.* 2007). Despite their body size, small-bodied fish often act as top predators in several freshwater ecosystems, such as intermittent rivers, headwater permanent streams, and ponds (Meyer *et al.* 2007; Reich *et al.* 2010; Brucet *et al.* 2012). However, these species are often overlooked in research and conservation management even when considered threatened, probably because they usually lack commercial value (Williams 2006; Braga *et al.* 2012; Saddlier *et al.* 2013) and inhabit ecosystems often neglected by conservation and management (Boix *et al.* 2012; Nikolaidis *et al.* 2013). Therefore, understanding the ecological consequences of the possible loss of small-bodied predatory fish should be a research priority.

Many freshwater systems, such as most forested headwater streams, are not autotrophic-based, but detritus-based systems where leaf-litter processing fuels food webs (Vannote *et al.* 1980; Graça & Canhoto 2006; Tank *et al.* 2010). The hypothesis that detritus-based food webs are less likely to be top down-controlled compared with grazing systems (Rosenfeld 2000; Johnson & Wallace 2005) was refuted decades ago by several studies that have demonstrated how predatory invertebrates can trigger trophic cascades in detritus-based streams (Obernborfer *et al.* 1984; Malmqvist 1993; Lecerf & Richardson 2011; Lagrue, Besson & Lecerf 2015). However, fish-induced trophic cascades in streams have been demonstrated more recently and are less common in the literature (Greig & McIntosh 2006; Woodward *et al.* 2008; Buria *et al.* 2010) with some studies finding inconsistent (Ruetz, Newman & Vondracek 2002) or no effects on leaf breakdown by predatory fish (Reice 1991; Rosenfeld 2000). The indirect decrease of leaf breakdown by predators can be density-mediated, through the reduction of shredder density, but also trait-mediated (Schmitz *et al.* 2004; Lagrue *et al.* 2015), if the foraging activity of shredders decreases due to the predator presence.

In addition, top predators can play a key role in nutrient recycling through the excretion and egestion of nutrients (Vanni 2002; Schmitz *et al.* 2010). Thus, top predators may contribute toward the bottom-up control of food webs. In freshwater systems, predatory fish can produce biogeochemical hotspots when they aggregate in local patches (McIntyre *et al.* 2008; Boulêtreau *et al.* 2011; Capps & Flecker 2013). Although the potential effects of predatory fish in leaf breakdown through nutrient recycling are practically unexplored, several studies posit that nutrient enrichment in streams can increase leaf fungal biomass and, consequently, enhance microbially mediated leaf breakdown (Gulis & Suberkropp 2003; Chung & Suberkropp 2008; Ferreira & Chauvet 2011b). Besides, higher nutrient availability in the stream water can increase leaf N content, thus modifying leaf-litter quality (Gulis & Suberkropp 2003). Therefore, given that fish extinctions can alter nutrient recycling in streams (McIntyre *et al.* 2007), fish absence may impact on leaf-litter processing through bottom-up control.

In the present study, we assessed the top-down impacts of an endangered small-bodied fish, *Barbus meridionalis* (A. Risso, 1827), to determine whether the loss of a top predator affects (1) leaf fungal biomass and leaf quality (i.e. leaf carbon:nitrogen (C:N) ratio and leaf toughness), (2) macroinvertebrate assemblages colonizing leaf packs, and (3) leaf breakdown rates. To achieve these objectives, we conducted a leaf bag experiment from late spring to early autumn in two adjacent reaches of an intermittent Mediterranean stream: a downstream reach with a population of *B. meridionalis* (control site) and an upstream fishless reach. We hypothesized that macroinvertebrate shredder populations are top-down controlled by *B. meridionalis,* thus the fishless reach would have higher shredder densities compared to the control reach and, consequently, faster macroinvertebrate mediated litter breakdown rates (Fig. 4.1). Moreover, we hypothesized that *B. meridionalis* can create a biogeochemical hotspot in intermittent streams during the dry season when this species aggregates in pools, as do other fish species (Aparicio & de Sostoa 1999). Hence, the absence of excretion and egestion by *B. meridionalis* may trigger a reduction in leaf fungal biomass, slowing down microbially mediated leaf breakdown.

Materials and methods

Study area

The study was carried out in the Castelló stream (lat 41°40′42″N, long 2°1′49″E; Altitude: 431 m a.s.l.) within the protected area of Sant Llorenç del Munt i l′Obac Natural Park (50 km inland from Barcelona city, NE Spain). This area is characterized by a calcareous geology and a Mediterranean climate, with mild winter and warm spring and summer. Rainfall is irregular and intense, occuring primarily in winter but also in spring and autumn, while summer is normally very dry. The protected area is dominated by Holm oak (*Quercus ilex* L.) and Aleppo pine (*Pinus halepensis*

Miller) forests and Mediterranean shrubs (for a detailed description see Bonada *et al.* 2007b, Verkaik *et al.* 2013b). The Castelló stream is a first order stream, tributary of the Ripoll river in the Besòs basin, and its catchment is mainly forested, with small areas of cereal cultivation (<15%). We selected two adjacent 100-m reaches in the stream, separated by <0.5 km: a downstream reach with a population of *B. meridionalis* and an upstream fishless reach. Both reaches were <2 m wide and <50 cm deep, with a bedrock stream bed and similar riparian vegetation and hydromorphological characteristics.

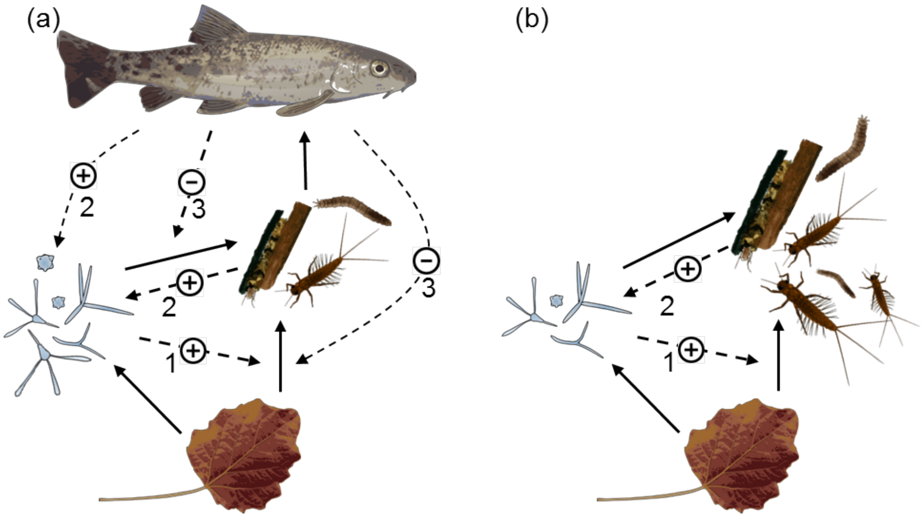


Figure 4.1 Trophic (solid) and non-trophic (dashed) interactions among the predatory fish, shredders, fungal decomposers, and leaf-litter in the presence (a) and absence (b) of the top predator. Non-trophic interactions include: (1) fungal facilitation of shredders through leaf conditioning, (2) fungal stimulation through nutrient excretion by fish and invertebrates, and (3) trait-mediated reduction of foraging activity. Adapted from Jabiol, McKie & Bruder (2013).

Species

Barbus meridionalis is endemic to NE Spain and SE France, and is currently listed as 'vulnerable' in the Spanish Red Book (Doadrio 2001), 'near threatened' in the IUCN, and included in Annexes II and V of the European Union Habitats Directive and in Appendix III ('protected fauna species') of the Bern Convention. Similar to other freshwater fish species, the list of threats include water pollution, water abstraction, dam construction, introduced species, and alterations in habitat (Doadrio *et al.* 2011; Maceda-Veiga 2013). This freshwater fish often acts as top predator in intermittent Mediterranean streams, where its local extinction may trigger a 'mesopredator release', a 'prey release', changes in the whole macroinvertebrate community composition, and a decrease in periphyton primary production (Rodríguez-Lozano *et al.* 2015b).

In August 2003, a wildfire burned a forested area of 4543 ha and, consequently, *B. meridionalis* was locally extinct in some streams, most likely due to water quality deterioration (Vila-Escalé *et al.* 2007a). The fish population has not recovered in those streams since then, possibly due to the presence of natural and human barriers downstream. The Castelló stream and its watershed were not affected by the wildfire, and its *Barbus meridionalis* population remains well preserved; as a result, this stream has been used as a control system for several research studies (e.g. Verkaik *et al.* 2013; Rodríguez-Lozano *et al.* 2015a).

Physicochemical measurements

We carried out physicochemical measurements throughout the litter decomposition experiment (n = 6). We measured dissolved O_2, pH, and conductivity *in situ* with a Multiline P4 WTW meter (YSI, Yellow Springs, OH, U.S.A.), and we estimated discharge from mean depth, transect width, and water velocity with a flow meter (miniAir, Schiltknecht, Gossau, Switzerland). We collected water samples by hand, reserved 50 mL for total organic carbon analysis (TOC), and filtered the rest of the water through GF/F Whatman filters to analyze dissolved organic carbon (DOC) and nutrients. TOC and DOC were determined in an automated N, C analyzer (multi N/C 3100, Analytik Jena, Jena, Germany). We measured ammonium (NH_4^+-N) and soluble reactive phosphorous (PO_4^{3-}-P) with spectrophotometric methods (Shimadzu UV-1201, Shimadzu, Tokyo, Japan) and nitrate (NO_3^--N) with ionic chromatography methods (UV/V Kontron model 332, Kontron AG, Zürich, Switzerland). Water temperature was recorded every 10 minutes throughout the leaf bag experiment using submersible temperature data loggers (HOBO Pendant, Onset Computer Corporation, Bourne, MA, U.S.A.).

Field experiment

We conducted the leaf bag experiment from late spring to early autumn 2012 (14 June 2012 – 05 October 2012), coinciding with the peak of in-stream leaf inputs (Rodríguez-Lozano *et al.* 2015a). Leaves of white poplar (*Populus alba* L.) were collected just after abscission, and air-dried at room temperature to constant mass. Leaf bags (15 x 20 cm) containing 3 g of leaves (SE = 0.06 g) were made of 2 mesh sizes: coarse (10 mm) and fine (250 µm). Coarse-mesh bags allowed macroinvertebrate colonization and also small fish individuals to enter the leaf bags, thus more closely simulating natural leaf-litter breakdown processes, whereas fine-mesh bags excluded virtually all fish and macroinvertebrates, thereby allowing us to assess the relative contribution of microbial (fungi and bacteria) activity to leaf breakdown (Young *et al.* 2008).

We deployed 30 bags of each mesh type in the control and fishless reaches in pools because leaves naturally accumulated in the pools of this stream. We placed 12 additional bags in the fishless reach for 24 h to correct for initial leaf mass losses resulting from leaching and accidental transport losses (Gessner *et al.* 1999). We removed 6 litter bags of each type (fine- and coarse-mesh) from each reach 5, 12, 26, 58, and 113 days after deployment. At retrieval, we placed litter bags individually in zip-lock bags and transported them in refrigerated containers to the laboratory, where we processed them immediately. We washed the material in each litter bag, collected invertebrates on a 250-µm sieve, and preserved them in 70% ethanol. For each litter bag, 8 leaf discs of 10-mm diameter were cut. A set of 5 discs were stored at -18ºC until ergosterol determinations (*n* = 6). The other 3 leaf discs were used to quantify leaf toughness (*n* = 3) or leaf C:N ratio (*n* = 3) (i.e. half of the samples where used for a measure, and the other half for the other, resulting in 3 replicates per treatment for both measures). The remaining leaf material was oven-dried (60°C, 72 h) and combusted (500°C, 12 h) to measure remaining ash-free dry mass (AFDM).

Laboratory analysis

Fungal biomass in leaf-litter was assessed through determination of ergosterol content, following Gessner (2005). The 5 frozen leaf discs of each leaf bag were freeze-dried, weighed to the nearest 0.1 mg and heated at 80ºC for 30 minutes with alkaline methanol to extract the lipids. Subsequently, extracts were purified using solid-phase extraction cartridges (Sep-Pak tC18, 500 mg, 3 cc, Waters Corporation, Milford, MA, U.S.A.). Ergosterol concentration was quantified by comparing absorbance at 282 nm after separation from other lipids by high-performance liquid chromatography (HPLC, JASCO, Easton, MD, U.S.A.; Column Gemini® 5 µm NX-C18 250 x 4.6 mm, Phenomenex, Torrance, CA, U.S.A.) with a standard of ergosterol (Sigma-Aldrich, St. Louis, MO, U.S.A.). Ergosterol content was converted to mycelium biomass using the average conversion factor of 5.5 mg of ergosterol per gram of mycelium (Gessner & Chauvet 1993). We standardized fungal biomass per gram of leaf-litter AFDM remaining in leaf bags. Leaf toughness was estimated by measuring the force needed to penetrate leaves using a penetrometer (punch diameter = 1.95 mm), following methods described by Graça & Zimmer (2005). For each treatment replicate, leaf toughness corresponded to the mean of 3 measurements on 3 distinct leaves. To determine the C:N ratio we grounded together 3 leaf discs of 3 distinct leaves for each treatment replicate. Subsequently, leaf C and N contents were quantified using a gas chromatograph coupled to a TCD detector after combustion at 1000ºC.

We counted macroinvertebrates, identified them to the lowest possible taxonomic level (usually genus), and measured them to the nearest mm. We classified taxa to functional feeding groups (FFG) following Tachet *et al.* (2010). We calculated individual biomass using published body length–dry mass equations (Dumont *et al.* 1975; Smock 1980; Meyer 1989; Benke *et al.* 1999; Miserendino 2001; Baumgärtner & Rothhaupt 2003; Ohta *et al.* 2011), except Hydracarina, Ostracoda, Cladocera, Oligochaeta, Nematoda, and Tardigrada for which we used biovolume data (Ramsay *et al.* 1997). We standardized macroinvertebrate abundance and biomass in coarse-mesh leaf bags per gram of remaining AFDM of leaf-litter in leaf bags. We also calculated macroinvertebrate mean and maximum body size (as body length) of each coarse-mesh litter bag.

Data analysis

All statistical analyses were performed using the programs R (version 2.15.2, R Project for Statistical Computing, Vienna, Austria) and SPSS (version 21.0, IBM Corp., Armonk, New York, U.S.A.). We compared mean daily water temperature and other physicochemical variables between control and fishless reaches, over the experimental period with one-way ANOVAs.

We used an exponential decay model (Bärlocher 2005) to quantify leaf breakdown rates:

$$M_t = M_0 e^{-kt} \qquad \text{(Eq. 4.1)}$$

where M_t is the leaf-litter AFDM at time t, M_0 is the initial AFDM corrected for leaching and transportation mass losses, $-k$ is the decomposition rate, and t is the time in days. To test for significant differences in leaf decomposition rates among sites and mesh sizes, we conducted an analysis of covariance (ANCOVA) on ln(x)-transformed AFDM remaining (dependent variable), with site and mesh size as fixed factors and time (d) as a covariate (Zar 2010). We adjusted α levels for pairwise comparisons of decomposition rates between all sites and mesh sizes with Bonferroni corrections. We corrected for temperature effects on leaf breakdown rates, by repeating the analyses using degree days (dd) instead of days as the covariate. We calculated degree days as mean daily temperatures accumulated by each sampling day (Minshall *et al.* 1983; Irons III *et al.* 1994; Menéndez *et al.* 2003). Similarly, to detect differences in leaf fungal biomass, toughness, and C:N ratio we conducted ANCOVA with site and mesh size as fixed factors and time as a covariate.

Nonmetric multidimensional scaling (NMDS) ordination was employed to examine the similarity in the macroinvertebrate composition of the coarse-mesh bags. To test for differences in the macroinvertebrate community composition between reaches, we used permutational multivariate analysis of variance (PERMANOVA, 'Adonis' function in R) on the Bray-Curtis distance matrix, after the log(x)-transformation of the macroinvertebrate biomass data. Subsequently, we used indicator species analysis, using 'IndVal' test in R, to identify which macroinvertebrate taxa of the coarse-mesh bags could serve as indicator for *B. meridionalis* presence or absence.

The 'IndVal' test calculated the indicator value for each taxon, combining measurements of taxon specificity to each reach with taxon fidelity within each reach (Dufrêne & Legendre 1997). The significance of 'IndVal' measures was tested using the Monte Carlo test with 9999 permutations. To test for significant differences in total macroinvertebrate abundance and biomass in coarse-mesh leaf bags between reaches, we performed ANCOVA with site as a fixed factor and time as a covariate. We repeated the analysis for each macroinvertebrate FFG, and applied Bonferroni corrections to control for comparison-wise error. We also tested for differences between reaches in mean and maximum body size of macroinvertebrates in coarse-mesh bags.

Results

Both reaches had alkaline pH, low discharge, and low nutrient concentrations during the study period (Table 4.1). Mean daily water temperature was similar between reaches ($F = 3.57$, $p = 0.06$). Reaches only differed in dissolved oxygen levels, which were slightly higher in the fishless than in the control reach ($F = 21.65$, $p < 0.001$). During the leaf bag experiment, the stream was in an oligorheic aquatic state (Gallart *et al.* 2012), i.e. pools were the dominant mesohabitat but still connected by thin water threads (flow <1 L s^{-1}).

Table 4.1 Mean (± 1 SE) values of characteristics of the control and the fishless reaches during the litter decomposition experiment (14 June 2012 – 05 October 2012).

	Control	Fishless
Discharge (L s^{-1})	0.7 ± 0.1	0.6 ± 0.2
Water temperature (ºC)	6.82 ± 0.05	6.60 ± 0.04
pH	8.16 ± 0.06	8.22 ± 0.05
Conductivity (µS cm^{-1})	478 ± 5	474 ± 20
DO (% saturation)	85.7 ± 0.9	95.1 ± 0.1
NH_4^+-N (µg L^{-1})	50 ± 7	48 ± 1
NO_3^--N (µg L^{-1})	102 ± 16	97 ± 27
PO_4^{3-}-P (µg L^{-1})	22 ± 4	28 ± 1
TOC (mg L^{-1})	3.59 ± 0.21	3.60 ± 0.24
DOC (mg L^{-1})	3.06 ± 0.11	3.16 ± 0.18

Poplar-leaf mass loss fit an exponential model with a similar adjust for days and degree days (Table 4.2). Litter mass loss over time (i.e. without temperature correction) was faster in the fishless compared to the control reach ($F = 4.10$, $p = 0.04$; Fig. 4.2a). Litter decomposition rate also differed between mesh sizes ($F = 28.03$, $p < 0.001$; Fig. 4.2a), and a significant interaction between both factors was detected ($F = 15.14$, $p < 0.001$). Using accumulated heat (degree days) instead of time (days) as independent variable, leaf decomposition rate also differed due to *B. meridionalis* presence ($F = 9.41$, $p = 0.003$; Fig. 4.2b) and mesh size ($F = 27.90$, $p < 0.001$), with a significant interaction between factors ($F = 15.08$, $p < 0.001$). Pairwise comparisons showed differences due to fish presence for coarse-mesh bags ($F = 26.90$, $p < 0.001$), but not for fine-mesh bags ($F = 1.08$, $p = 0.30$). Litter decomposition for coarse-mesh bags was faster compared to fine-mesh bags only in the fishless reach (control: $F = 1.84$, $p = 0.18$; fishless: $F = 31.06$, $p < 0.001$).

Poplar leaf toughness (Fig. 4.3a) decreased continuously over time ($F = 296.67$, $p < 0.001$), and was lower in fishless than control reach leaves ($F = 4.212$, $p = 0.04$). Mesh size had no effect on leaf toughness ($F = 1.94$, $p = 0.17$). Leaf fungal biomass (Fig. 4.3b) was higher in the control than in the fishless reach ($F = 5.45$, $p = 0.02$) and in the coarse-mesh than in the fine-mesh bags ($F = 4.30$, $p = 0.04$). No significant interaction between factors was found ($F = 0.79$, $p = 0.28$). Leaf C:N ratio (Fig. 4.3c) decreased over time ($F = 48.21$, $p < 0.001$), but no effect of *B. meridionalis* presence nor mesh size was detected ($p > 0.6$).

Table 4.2 Mean (SE) leaf-litter breakdown rates ($-k$) and R^2 values for regressions of ln(x)-transformed leaf mass remaining vs time in days (d) and cumulative degree days (dd).

		d			dd		
Site	Mesh	$-k$	SE	R^2	$-k$	SE	R^2
Control	Coarse	0.00786	0.00036	0.94	0.00115	0.000050	0.95
Control	Fine	0.00710	0.00033	0.94	0.00104	0.000047	0.95
Fishless	Coarse	0.01100	0.00031	0.98	0.00164	0.000047	0.98
Fishless	Fine	0.00627	0.00029	0.95	0.00093	0.000043	0.94

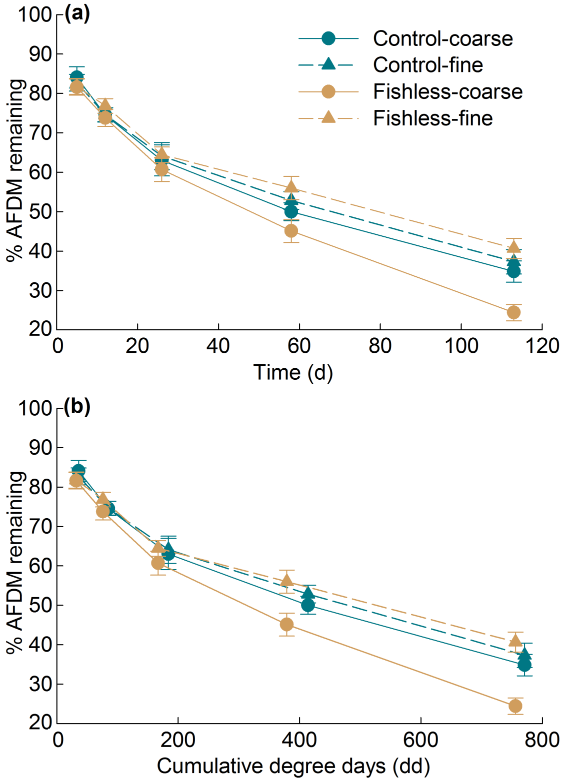


Figure 4.2 Mean (±1 SE; *n* = 6) % initial *Populus alba* leaf-litter mass remaining in coarse- and fine-mesh bags at the control and the fishless reaches over 113 days expressed over time (a) and over cumulative degree days (b).

The NMDS plot based on biomass data of macroinvertebrate taxa provided an interpretable two-dimensional ordination of the macroinvertebrate assemblages of the coarse-mesh leaf bags (Fig. 4.4). The taxonomic composition of the macroinvertebrate assemblages differed significantly depending on *B. meridionalis* presence (Adonis, $F = 30.86$, $p < 0.001$). Macroinvertebrate composition also changed over time ($F = 10.03$, $p < 0.001$), with a significant interaction between factors ($F = 5.76$, $p < 0.001$): the differences in macroinvertebrate assemblages between reaches decreased during the leaf breakdown process (Fig. 4.4). Eight taxa were identified as indicators of fish presence and six taxa of fish absence (Table 4.3). The shredders *Habrophlebia* sp. and Stratiomyidae, as well as several scrapers (i.e. *Gyraulus* sp., *Radix* sp., and *Ancylus* sp.), were characteristic of the leaf bags at the fishless reach. The shredder *Thraullus bellus* and the Orthocladiinae family (scrapers) were characteristic of *B. meridionalis* presence. Other shredders, such as *Mystacides* sp., Limnephilidae, and Tipulidae were not indicators of top predator presence or absence.

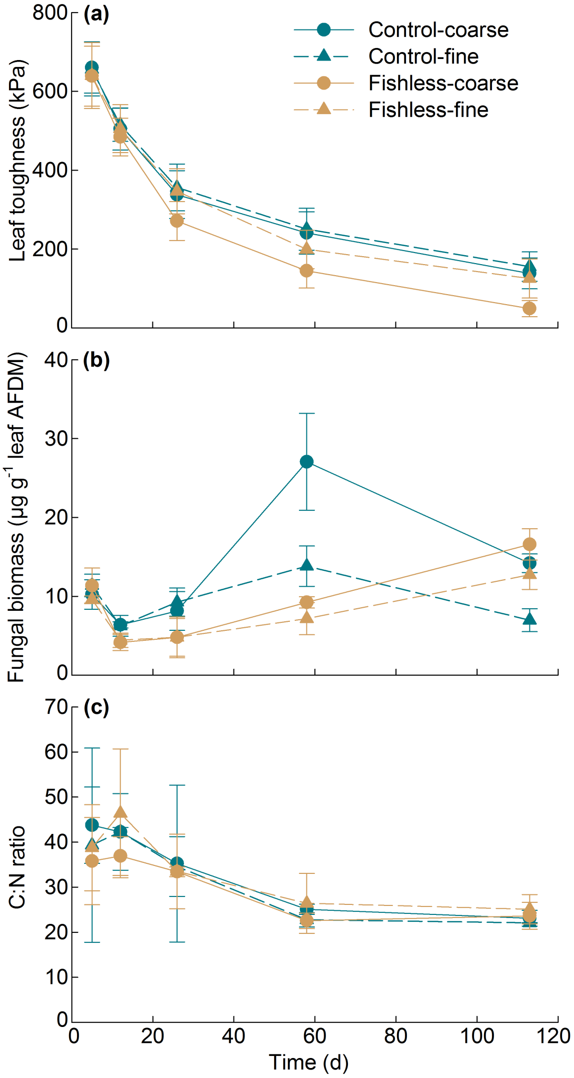


Figure 4.3 Mean (±1 SE) leaf toughness (*n* = 6) (a), leaf fungal biomass (*n* = 3) (b), and C:N ratio (*n* = 3) (c) in coarse- and fine-mesh bags at the control and the fishless reaches over the experimental period.

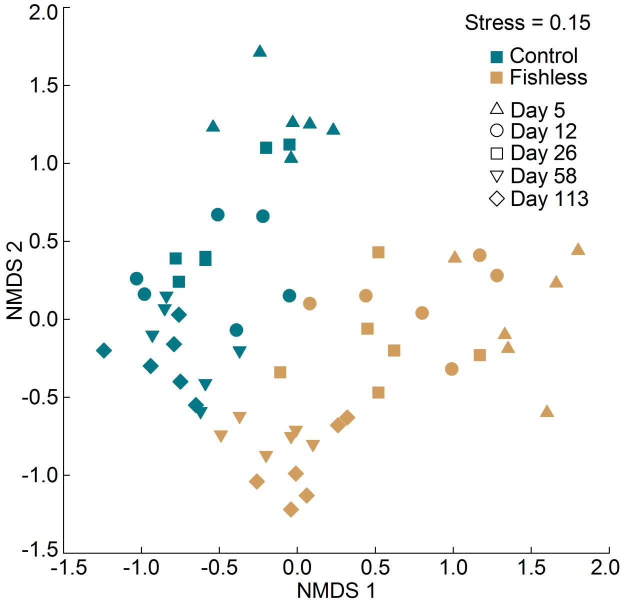


Figure 4.4 Nonmetric multidimensional scaling (NMDS) ordination of macroinvertebrate assemblages in coarse-mesh bags. Colors distinguish *Barbus meridionalis* presence and absence and different symbol shapes denote different sampling dates.

Table 4.3 Macroinvertebrate taxa detected as significant indicators for the coarse-mesh leaf bags in the control and the fishless reaches. T – Treatments: 1 = control, 2 = fishless. IndVal – indicator value. *p* – its respective *p*-value.

Taxa	T	IndVal	*p*
Cladocera	1	80.17	<0.001
Caenis sp.	1	73.44	<0.001
Oligochaeta	1	62.00	0.003
Corynoneura sp.	1	60.17	0.010
Orthocladiinae	1	59.00	0.002
Ceratopogoninae	1	51.82	0.003
Thraulus bellus	1	23.33	0.011
Planaria torva	1	20.00	0.024
Radix sp.	2	79.85	<0.001
Gyraulus sp.	2	72.89	<0.001
Habrophlebia sp.	2	56.16	<0.001
Copepoda	2	46.56	0.008
Stratiomyidae	2	29.95	0.003
Ancylus fluviatilis	2	26.67	0.005

Total macroinvertebrate abundance in the coarse-mesh bags did not differ between reaches ($F = 1.04$, $p = 0.31$), while total macroinvertebrate biomass (Fig. 4.5a) was higher in the fishless reach compared to the control reach ($F = 5.01$, $p = 0.03$). Mean body size ($F = 6.00$, $p = 0.01$) and maximum body size ($F = 2.00$, $p < 0.001$) were higher in the coarse-mesh leaf bags at the fishless than at the control reach. In terms of functional feeding groups (FFG), the abundance and biomass of shredders and scrapers (Fig. 4.5b-c) was higher in the coarse-mesh bags of the fishless reach compared to the control reach, but the abundance and biomass of gathering and filtering collectors (Fig. 4.5e-f) was greater in the control than in the fishless reach ($F = 6 - 36$, $p = 0.01$ to < 0.001). Abundance and biomass of predatory invertebrates in coarse-mesh bags did not differ between reaches ($p > 0.9$; Fig. 4.5d).

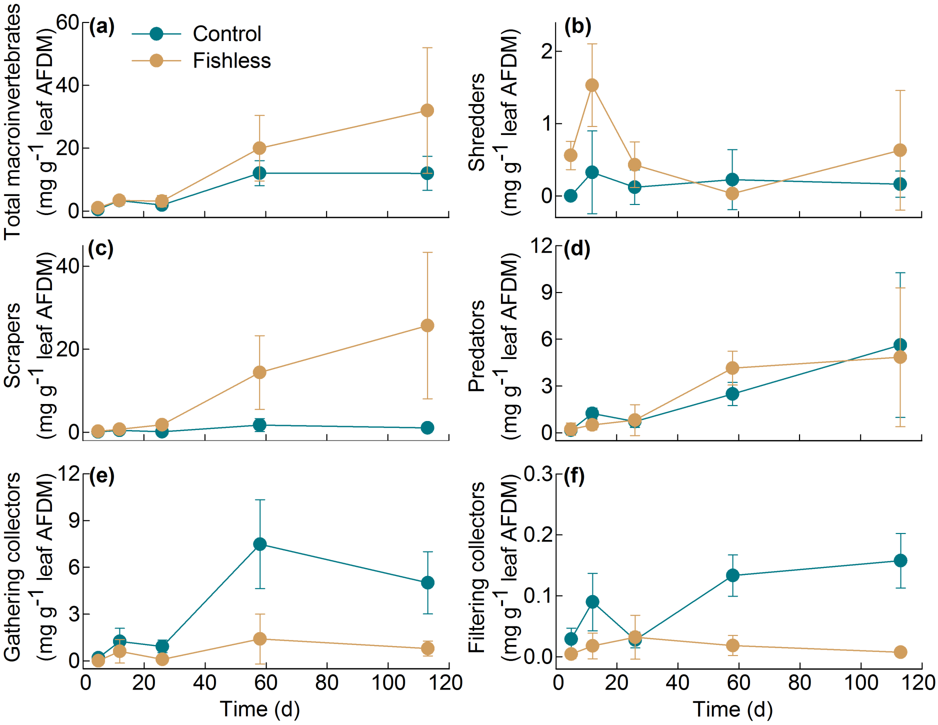


Figure 4.5 Mean (±1 SE; $n = 6$) biomass (mg g^{-1} remaining leaf ash-free dry mass [AFDM]) of total invertebrates (a), shredders (b), scrapers (c), predators (d), gathering collectors (e), and filtering collectors (f) in coarse-mesh bags at the control and the fishless reaches over the experimental period.

Discussion

Our results show that an endangered small-bodied fish triggered a trophic cascade in a detritus-based stream. Despite the growing recognition of the importance of intermittent streams, no published study has assessed trophic cascades of predatory fish over leaf decomposition in these systems. Overall leaf breakdown (i.e. coarse-mesh bags) was faster in the fishless than in the control reach, but microbially mediated decomposition (i.e. fine-mesh bags) did not differ due to top predator presence-absence. Therefore, the difference in leaf breakdown between reaches can be attributed entirely to differences in macroinvertebrate performance. We isolated the effects of macroinvertebrates on leaf breakdown rates by subtracting *k*-values in fine-mesh bags from those in coarse-mesh bags. These calculations showed that the macroinvertebrate mediated leaf breakdown rate was 6.2 times higher in the fishless than in the control reach (i.e. 0.00473 vs 0.00076 when expressed per day). This difference could be caused by intersite differences in shredder density and biomass in coarse-mesh bags, which were significantly higher in the fishless than in the control site. Scraper density and biomass were also higher in coarse-mesh bags in the fishless stream, which could also have contributed toward increasing leaf breakdown since scrapers, such as gastropods, can also have a positive effect on leaf breakdown rates (Costantini & Rossi 2010; Treplin & Zimmer 2012). These differences in the density and biomass of shredders and scrapers in coarse-mesh bags between reaches can be attributed to the top-down control by *B. meridionalis* in the control reach. Previous research has shown that this fish species can trigger a trophic-cascade on periphyton primary production (Rodríguez-Lozano *et al.* 2015b) and may feed preferentially on macroinvertebrate shredders (see Chapter 3).

The macroinvertebrate contribution to leaf breakdown was negligible in the control reach (i.e. no significant differences in breakdown rates between fine- and coarse-mesh bags), although shredders and scrapers were present in coarse-mesh bags. This suggests that *B. meridionalis* presence not only had a density-mediated impact, but also a trait-mediated, reducing macroinvertebrate feeding activity on leaf-litter. Schmitz *et al.* (2004) suggested that trait-mediated responses of prey to predators ultimately determine trophic cascades, but studies on behaviorally mediated effects of predatory fish on detritus-based systems are scarce and their results are equivocal (Greig & McIntosh 2006; Boyero *et al.* 2008; Jabiol *et al.* 2013). Several laboratory studies have found that shredders may reduce their activity in presence of fish chemical cues (Abjörnsson *et al.* 2000; Boyero *et al.* 2008), but only in some cases it led to a reduction in leaf breakdown (Short & Holomuzki 1992; Rezende *et al.* 2015). The trait-mediated response of prey could depend on the predator-prey pair as a function of the prey vulnerability to the predator and the existence of other antipredator strategies (Schmitz *et al.* 2004; Jabiol *et al.* 2013).

In our study, *B meridionalis*' top-down role over macroinvertebrates was not limited to the reduction of some taxa density, but it changed the whole macroinvertebrate assemblage associated to leaf bags. For instance, *B. meridionalis* favored filtering collectors, such as cladocerans, which may be caused by the fish foraging movements that can dislodge and resuspend deposited sediments (Gelwick, Stock & Matthews 1997). The observed decrease in macroinvertebrate mean and maximum body sizes in top predator presence was probably caused by these changes in community composition (i.e. several small invertebrate taxa were favored, such as cladocerans and Orthocladinae), and by the size-selectivity feeding of *B. meridionalis* (Chapter 3). The changes in the assemblage composition can affect leaf breakdown rates if the favored taxa cannot functionally compensate the reduction or loss of other taxa (Ernest & Brown 2001; Rodríguez-Lozano *et al.* 2015b). Greig and McIntosh (2006) found no differences in total shredder biomass among streams with and without brown trout, but higher leaf decomposition rates in fishless streams; it was explained by changes in the composition of the shredder assemblages as an efficient obligate shredder was only present in fishless streams. This suggests that the top-down impact of a predatory fish on leaf decomposition can depend on the interaction strength between shredder prey and leaf-litter; shredder identity being a key factor.

In addition, fish presence positively affected leaf fungal biomass in both fine- and coarse-mesh bags, but did not lead to an increase in microbially mediated leaf breakdown (i.e. no significant differences between reaches in leaf decomposition in fine-mesh bags). Moreover, fungal biomass was higher in coarse-mesh compared to fine-mesh bags, thus macroinvertebrates positively affected fungal biomass too, improving micro-environmental conditions for fungal growth in leaf bags. These results suggest that, as we hypothesized, leaf fungal biomass was bottom-up stimulated through nutrient recycling by both the top predator and macroinvertebrates (see Fig. 4.1). In general, the role of predators in the microbial communities involved in leaf-litter processing in freshwater systems is highly unknown (but see Majdi *et al.* 2015).

In a lacustrine environment, fish presence increased leaf fungal biomass in summer but not in winter, when fish density was lower (Mancinelli, Costantini & Rossi 2007). In our experiment, although stream pools did not disconnect completely, fish aggregated in pools at high densities which may have created biogeochemical hotspots (McIntyre *et al.* 2008; Boulêtreau *et al.* 2011; Capps & Flecker 2013). Because the experiment was carried out in summer, the water temperature was the highest during the year (mean daily temperature was 6.71ºC with maximums >10ºC) which could have a synergistic effect with nutrient recycling (Ferreira & Chauvet 2011b). To our knowledge, the present study is the first to have specifically explored the impact of a predatory fish in leaf fungal biomass in a stream.

Although *B. meridionalis* presence increased leaf fungal biomass, it had no impact on streamwater TOC, DOC, and nutrient concentrations. This suggests that top predator impact on nutrient recycling was produced through excretion of metabolic waste, thus labile carbon and nutrients entered in the fast cycle in inorganic forms and were rapidly taken up by the microbial assemblages, moving directly to the stream food web (Schmitz *et al.* 2010; Atkinson, Kelly & Vaughn 2014). Microbial assemblages in leaf-litter can obtain nutrients from the leaves and from the streamwater. The proportion of streamwater N and C that is used by microbial assemblages in leaf-litter increases during the decomposition process (Pastor *et al.* 2014), and in the case of N can reach >80% (Cheever *et al.* 2013). Moreover, our study site was an oligotrophic stream and *Populus alba* leaves were initially not rich in N (1.3 ± 0.1%), thus microbial decomposers were probably limited by nutrients and labile C being more easily influenced by fish and macroinvertebrate effects on nutrient recycling (Ferreira, Gulis & Graça 2006b; Benstead *et al.* 2010; Vanni 2010). In fact, the LINX II project (Mulholland *et al.* 2008) demonstrated that the efficiency of N biotic uptake from streamwater increased as N concentration in water column decreased.

Leaf C:N ratio decreased over time, most likely because of microbial immobilization of nitrogen (Ferreira *et al.* 2006b). *Barbus meridionalis* had no impact on leaf C:N ratio despite its positive effect on leaf fungal biomass. Previous research has shown that nutrient enrichment may increase leaf fungal biomass, while having no impact on N leaf content (Ferreira *et al.* 2006b). Robinson and Gessner (2000) showed that nutrient addition increased N and P in leaves, but the effect disappeared after three weeks. Regarding leaf toughness, it followed a similar trend than leaf mass loss. Therefore, although initial leaf toughness can be a significant predictor of leaf breakdown rates and leaf consumption by invertebrates and microorganisms when comparing different leaf species (Graça & Zimmer 2005), leaf toughness seems to be a suitable alternative measure for assessing leaf breakdown (Young *et al.* 2008).

In conclusion, our study proves that the loss of an endangered small-bodied fish may lead to deep ecosystem changes in a detritus-based stream. The decrease in vertical diversity led to the simplification of the functional system complexity. Top predator absence triggered a density-mediated trophic cascade, accelerating leaf decomposition rates. Fish presence also changed the composition of macroinvertebrate assemblages, reduced invertebrate mean and maximum body sizes, and most likely reduced the foraging activity of some invertebrates, which may have contributed to the trophic cascade. Moreover, fish extirpation indirectly decreased leaf fungal biomass, probably caused by the loss of the bottom-up stimulation through nutrient recycling by fish. The key role of small-bodied fish in stream food-webs may increase during the dry season due to their aggregation in pools, particularly in intermittent streams. This is highly relevant because, although intermittent streams have been neglected by research and conservation (Nikolaidis *et al.* 2013), these systems comprise approximately one half of the global river network and are projected to increase due to climate change and water abstraction for human use (Carlisle *et al.* 2011).

Our study also exemplifies that the ecosystem impacts of top predator loss are not limited to top-down trophic cascades. In this sense, the lack of studies to have directly traced and quantified the contribution of consumer nutrient remineralization to food webs (but see Atkinson *et al.* 2014) shows our lack of awareness of the potential importance of top predators in this ecosystem process. Finally, given the high extinction risk of small-bodied freshwater fish species, we think that conservation efforts must be undertaken because of both their inherent value and the major ecosystem changes that may cause their loss.

General discussion and conclusions

The overarching goal of this PhD thesis was to assess the long-term effects of a wildfire in Mediterranean streams. We have focused on the effects of fire in stream food webs through two fire legacies: (1) the reduction of the riparian canopy and (2) the extirpation of the top predator. Consequently, one of the main conclusions of this PhD thesis was that past fires may have current influence on the structure and function of Mediterranean streams. Previously to this PhD thesis, research on fire effects on streams have mainly conducted in the short- and mid-term (<5 years), thus the long-term approach of this PhD thesis is one of its main strengths.

During the development of this PhD thesis, the research on fire effects on aquatic systems has grown, as showed by the special issue published in the *Freshwater Science* Journal in December 2015 (Bixby *et al.* 2015). The rising interest in this topic relies on the projected increase in fire frequency and intensity (Bowman *et al.* 2011; Dury *et al.* 2011; IPCC 2014). The attention over the ecology of intermittent streams is also under expansion (Leigh *et al.* in press). Moreover, the top predator decline has been considered the main current threat for ecosystems worldwide (Estes *et al.* 2011). Therefore, this PhD thesis interconnected several contemporary topics in ecology.

This section aims at putting in a wider context some aspects that have arose along this PhD thesis. We will discuss about (1) the importance of past fires for streams, (2) the role of apex consumers in intermittent streams, (3) the research on the effects of nonrandom extinctions with a trophic web approach, and (4) the introduction of non-native species within the context of the current biodiversity loss at global scale.

The stream and its valley, and the history of its valley

Although fire is a pulse disturbance, we demonstrated that its effects on aquatic ecosystems can be long lived: 'the ghost of past fires'. The results of Chapter 1 showed how the removal of the riparian canopy by fire reduced litterfall inputs and accelerated leaf breakdown rates eight years after fire. Therefore, Chapter 1 contributed substantially to a very limited literature on fire effects on detrital dynamics and leaf breakdown rates (Koetsier *et al.* 2010; Jackson *et al.* 2012; Vaz *et al.* 2015). It should be noted that this limited literature has been published during the development of this PhD thesis, thus information on this topic was almost inexistent before. In general, a substantial amount of literature has focused on how fire affects state variables (i.e. physicochemical parameters and the abundance of aquatic organisms), while rate variables have received little attention (but see Robinson *et al.* 2005; Betts & Jones Jr. 2009; Koetsier *et al.* 2010; Diemer *et al.* 2015; Klose, Cooper & Bennett 2015). Consequently, more research is needed to complement the limited available data on fire effects on ecosystem processes (e.g. leaf breakdown, stream metabolism, nutrient spiraling, nutrient uptake and limitation). More importantly, Chapter 1 demonstrated that fire effects on detrital dynamics can be long lived in the Mediterranean region.

Sant Llorenç del Munt wildfire extirpated fish populations from the streams and rivers draining burned catchments. Nowadays, more than a decade after the fire, some streams that were inhabited by *Barbus meridionalis* populations remain fishless. The results of this PhD thesis demonstrated that the local extinction of this top predator due to the wildfire may lead to complex ecosystem consequences at present. Top predator extirpation can modify both structure and function through changes in trophic and non-trophic interactions, simplifying the ecological networks in the affected streams.

Our study case is not singular, wildfires usually extirpate or dramatically reduce fish populations (Rinne 1996; Gresswell 1999; Burton 2005; Sestrich *et al.* 2011; Whitney *et al.* 2015). Fish kills are thought to result from hypoxia, contaminants introduced with fire-fighting activities, and elevated concentrations of toxic compounds (i.e. NH_4^+, PAHs, trace metals…). However, the ecosystem consequences of fish extirpation by fire and, in general, the ramifying indirect effects of fire on biological interactions are almost unexplored. After the Jesusita fire in Santa Barbara (California, U.S.A.), southern California steelhead trout were extirpated in burned basins (Cooper *et al.* 2015). Predatory invertebrate abundances were low in these streams before fire, but they increased after trout were locally extinct, reaching densities comparable to those in unburned fishless streams, while remaining at low densities in unburned trout streams. These results are consistent with our findings in Chapter 2 of 'mesopredator release' after *B. meridionalis* extirpation. Future research must address the knowledge gap on fire effects in trophic webs.

The duration of the cascading effects of fish extirpations caused by fires may depend on the time necessary for the recovery of fish populations. Fish populations can recover quickly if no barriers to fish immigration are present (Gresswell 1999), which is common in temperate forests on public lands. However, in Mediterranean streams many human-made barriers block fish migrations as consequence of the high population density in the Mediterranean basin (Verkaik *et al.* 2013a) and because the presence of man since >1000 years ago (e.g. medieval water mills). In fact, human population density and economic production have been related with the loss of fish biodiversity (Clausen & York 2008). It illustrates how watershed disturbances (i.e. wildfire) may interact with human drivers that affect river health (e.g. dams), being this interaction the key determinant of the legacy duration.

The importance of catchment disturbance legacies for aquatic ecosystems has been previously demonstrated for past land use changes. For instance, composition and diversity of macroinvertebrates and fish in southern Appalachian streams were best predicted not by current catchment land use (mainly secondary forest landscapes), but by watershed land use over five decades earlier (agricultural landscapes) (Harding *et al.* 1998). Similarly, Surasinghe & Baldwin (2014) showed that stream salamander diversity and community structure in southeastern U.S.A. was negatively impacted by historical land uses (particularly row-crop agriculture). The interpretation of the landscape influence in aquatic ecosystems is further complicated when cycles of change occur (Allan 2004), such as when agricultural land reverts to forest or forest land is burned and a secondary succession starts. Therefore, despite fire is a pulse disturbance, a dynamic process along decades just starts when fire is extinguished.

Ecological interpretation of mechanisms driving community assemblages and ecosystem functioning (e.g. to assess ecosystem health) should be undertaken with caution, because past fires and past land uses could be responsible for current patterns of species distributions and ecosystem processes in streams and rivers. Consequently, recognition of the importance of environmental history and its legacies in freshwater ecosystems is essential to better understand current conditions and to implement adequate conservation decisions.

Finally, it is worth noting that, although fire is considered a natural disturbance when is not directly provoked by humans, current fire regimes are shaped by human activity (Bowman *et al.* 2011; Dury *et al.* 2011; IPCC 2014). Therefore, the modification of natural disturbance regimes, especially fire, must be considered a human activity that exert a widespread impact on terrestrial and aquatic ecosystem worldwide (Foster *et al.* 2003). Consequently, the effects of fires in freshwater ecosystems are not a natural ecological dynamic, but an indirect consequence of human changes in land uses and human greenhouse gasses emission through animal production and fossil fuels use (Nordgren 2012; Machovina, Feeley & Ripple 2015).

Food webs in intermittent streams: from individuals to 'riverscapes'

The results of this PhD thesis, together with other studies done in temporary salt marshes (Compte *et al.* 2012) and permanent headwater streams (Woodward *et al.* 2008; Gido *et al.* 2010), showed that the effects of the loss of small-bodied fish are equivalent to local extinctions of larger apex consumers in other ecosystems (e.g. the arctic fox, wolf, jaguar, sea otter or large reef fish (Beschta & Ripple 2009; Estes *et al.* 2011; Ripple *et al.* 2014)). During the development of this PhD thesis, Boersma *et al.* (2014) showed that the extirpation of an invertebrate top predator (the hemipteran *Albedus herberti*) in desert intermittent streams led to 'mesopredator release' and reduced detritivore abundances. Therefore, both intermittent streams fishless and with predatory fish can be affected by the consequences of the loss of apex consumers.

Currently, our research in this topic has continued through the analysis of the stream food web using stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) in the reaches studied in Chapter 4. Our analyses confirmed that *B. meridionalis* acts as top predator in the stream (i.e. highest $\delta^{15}N$ values). We observed changes in the isotopic signal for some macroinvertebrate populations depending on the presence/absence of the top predator. In general, the $\delta^{15}N$ signal of predatory invertebrates was lower in the presence of *B. meridionalis*, which suggested that mesopredators decreased their predation rate on other invertebrates and increased their feeding on primary sources. These changes in mesopredators' diets may be an adaptation to reduce their predation risk and/or the competition with the top predator. Changes on the trophic position of macroinvertebrates due to the presence of small-bodied fish have been previously observed (Compte *et al.* 2012). Besides, isotopic signals of mesopredators were more variable at top predator presence. Because we measured isotopic values of predators at individual level, these results indicated higher diet variability among individuals within macroinvertebrate populations when *B. meridionalis* was present. Thus, these results also illustrated that trophic interactions occur between individual organisms, not at species level.

In this sense, the use of functional traits can improve our understanding of predator-prey interactions at individual level. In Chapter 3, we explored the benefits of using a common selectivity index in a trait-based perspective, through the study of the feeding ecology of *B. meridionalis*. In brief, Chapter 3 showed that morphological and behavioral traits of the potential prey may explain prey vulnerability to predation. One of the findings was that shredders were highly selected by the top predator, which suggested that *B. meridionalis* mainly forages in leaf-litter habitat patches.

Through the incorporation of landscape ecology ideas into rivers, such as the development of the 'patch dynamics concept' of stream ecology (Pringle *et al.* 1988; Townsend 1989), ecologists started to view rivers as 'riverscapes', i.e. as complex mosaics of habitat types and environmental gradients with high spatial complexity (Fausch *et al.* 2002; Allan 2004). Patchy environments within 'riverscapes' can produce 'environment-mediated interaction modifications' (Wootton 2002), such as a reduction of predation risk on prey by macrophytes when used as refuge (Diehl 1992). In order to assess if the influence of *B. meridionalis* in macroinvertebrate assemblages depends on the benthos patches within streams, we analyzed the macroinvertebrate communities of the reaches studied in Chapter 4, sampling separately the main three habitats: fine sediment, leaf-litter, and macrophytes (Belmonte-Viudez 2013). This study showed that top predator presence shaped macroinvertebrate assemblages, while habitat type had lower influence. Macroinvertebrate communities associated to leaf-litter were the most affected compared to the other two habitats (i.e. sediment and macrophytes), which agreed with Chapter 3 results. Significant differences were found between reaches for the macrophytes substrate, which supported that small-bodied fish can move and prey within this habitat (Meerhoff *et al.* 2007).

The results of this PhD thesis evidenced that the loss of small-bodied freshwater fish in intermittent streams may affect ecosystem structure and functioning during the oligorheic aquatic state (i.e. when pools are dominant, but still connected by thin water threads; Gallart *et al.* 2012). Future research is needed at larger spatial and temporal scales to integrate the effects of the hydrological variability of these systems. Top predators' role in intermittent rivers could vary quantitatively and qualitatively through changes in aquatic states.

Based on this PhD thesis results and our ongoing research, we recommend that reintroduction programs be considered for small-bodied fish in intermittent streams, where species such *B. meridionalis* had become locally extinct. Reintroduction programs would allow not just for recovery of endangered species populations, but for the restoration of the ecosystem. Therefore, reintroductions should be considered within an ecosystem restoration framework. In this sense, it is worth noting that current environmental policies often overlook small aquatic systems (Boix *et al.* 2012; Nikolaidis *et al.* 2013) where small-bodied fish species may act as top predators. Streams with catchments <1000 ha and ponds are not covered by the Water Framework Directive (European Commission 2000) and, thus, European governments are not obligated to achieve the objective of good ecological status for these ecosystems. Consequently, a first step for the effective protection of endangered small-bodied fish should be to include the ecosystems they inhabit in conservation policies.

Biodiversity-ecosystem function relationship and nonrandom extinctions

Motivated by the global biodiversity loss, a new subfield of ecology pop up two decades ago: the study of the functional consequences of biodiversity loss in ecosystems (i.e. biodiversity-ecosystem function relationship, B-EF) (Loreau *et al.* 2001). Most studies in B-EF relationship have not reproduced any sequence of species loss, instead have performed experiments using synthetic communities in which combinations of species or functional types are artificially assembled to represent different diversity levels (i.e. synthetic-assemblage experiments). Therefore, these studies relay on the unrealistic assumption that all species are equally susceptible to extinction (Duffy 2002; Finke & Snyder 2010). Hence, these synthetic–assemblage experiments are useful to answer questions about species richness *per se*, but they are hardly applicable to predict functional consequences of actual biodiversity loss, being non relevant for conservation objectives (Loreau *et al.* 2001; Diaz *et al*. 2003).

Initially, synthetic-assemblage experiments have focused in terrestrial ecosystems (primarily grasslands) and on aquatic microbial microcosms (Duffy 2002). Moreover, they have usually considered just a single trophic level (Petchey *et al.* 2004), overlooking trophic complexity (but see Jabiol *et al.* 2013). Vertical diversity (i.e. diversity across trophic levels) can alter B-EF relationships qualitatively, thus, the incorporation of interactions among multiple ecological groups is necessary in order to better understand actual B-EF relationships in nature (Duffy et al. 2007). Indeed, given that current biodiversity loss is biased to high trophic levels, resulting in trophic downgrading of most ecosystems (Duffy 2002; Estes *et al.* 2011), vertical instead horizontal diversity (i.e. diversity within a single trophic level) deserves special attention.

Alternatively to synthetic-assemblage experiments, removal experiments have been proposed (Diaz *et al.* 2003). Removal experiments are those in which the diversity of naturally assembled communities is manipulated by removing one or various components, allowing researchers to reproduce an expected pattern of species extinctions. Removal experiments may complement synthetic-assemblage experiments in studying the relationship between biodiversity and ecosystem functioning. More importantly, they are more useful for predicting actual ecosystem consequences of species loss, and thus, for making management decisions when relevant.

Part of this PhD thesis (Chapters 2 and 4) has focused on the ecosystem impacts of the loss of an endangered species, *B. meridionalis*. The experiments were carried out from a food web approach in streams naturally inhabited (currently or in the past) by this species, and incorporated almost all ecosystem complexity, making them relevant for conservation. These chapters have assessed the effects of top predator loss on ecosystem functioning, (i.e. periphyton primary production and leaf breakdown). Hence, this PhD thesis contributed to the knowledge regarding two current ecological questions (Sutherland *et al.* 2013): “How is ecosystem function altered under realistic scenarios of biodiversity change?” and “What are the ecosystem impacts of worldwide top predator declines?”.

Results of Chapter 2 showed that the role of the apex consumer was not functionally replaced by the remaining predatory species (Ernest & Brown 2001; Chalcraft & Resetarits 2003), despite of the increase in invertebrate predator abundance and richness. It suggested that the predator assemblage is more important than diversity *per se* (Finke & Denno 2005; Cardinale *et al.* 2006; Schneider & Brose 2013), and thus, the cascade strength depends on species identity (Borer *et al.* 2005). Several ecologists have highlighted that the observed effects of increasing biodiversity on ecosystem functioning in synthetic-assemblage experiments may be a probabilistic effect (called 'sampling effect') that consists in the greater chance for a more diverse assemblage of including one species with strong influence on ecosystem functioning, which will shape the overall B-EF relationship (Duffy 2002; Ives, Cardinale & Snyder 2004; Finke & Denno 2005; Cardinale *et al.* 2006; Schneider & Brose 2013). Therefore, the actual question would not be if biodiversity is linked to ecosystem functioning, but if endangered species are keystone species (i.e. those whose effects in the ecosystem are disproportionate to their abundance), and thus, if their loss will affect ecosystem functioning.

Research on how the loss of endangered species may affect ecosystem functioning is relevant for conservation as a justification to the public for protecting species at extinction risk. It highlights the ecological value of species, i.e. the value that a species has by virtue of the contribution it makes to the integrity (health, stability, or functioning) of the ecosystems of which it is part (Sandler 2010). This is particularly important because, currently, instrumental values of nature based on an anthropocentric view (e.g. the ecosystem services approach) have supplanted intrinsic and ecologic values, and most endangered small-bodied freshwater fish lack instrumental values (i.e. commercial, recreational).

As this PhD thesis showed, food webs provide a natural framework for understanding the ecological role of species and the mechanisms through which biodiversity and specific taxa influence ecosystem processes (Thompson *et al.* 2012). The artificiality and unsuitability of the historical separation between community ecology and ecosystem ecology, and therefore among ecologists, is now more evident than ever. Ecosystem ecologists can no longer ignore the composition of biological communities (usually considered black boxes) to study the fluxes of energy and materials, and community ecologists must take into account ecosystem processes to analyze the patterns of species richness and community assemblages in ecosystems. This PhD thesis, through a food web approach, was a little step to demolish this artificial division imposed by science history.

Species extinctions and introductions

Despite the loss of biodiversity at planetary scale, several researchers posit that biodiversity has increased at regional and local scales as a result of the introduction of non-native species (Sax & Gaines 2003). Consequently, several ecologists disagree with Estes *et al.* (2011) about that the global loss of biodiversity is causing the trophic downgrading of planet Earth and, alternatively, have defended that non-native species are functionally compensating the loss of apex consumers (Cucherousset, Blanchet & Olden 2012).

To uphold their opinion, Cucherousset *et al.* (2012) used freshwater fish as models. They analyzed the historical (i.e. current native species) and contemporary (i.e. current native and non-native species) trophic position of fish species in 13 watersheds. Their results showed that the maximum trophic position in these watersheds has increased (or no change), and the minimum trophic position has decreased (or no change), most likely because freshwater fish species have been introduced in both upper and lower trophic positions.

Results from this PhD thesis disagree with Cucherousset *et al.* (2012) results and conclusions for three reasons. First, they used current native species as a proxy of historical fish distributions in watersheds, thus they assumed no extinctions within these watersheds. This supposition ignores the own motivation of the analysis, the current biodiversity loss, and that freshwater fish are one of the most endangered groups worldwide (Jenkins 2003; Olden *et al.* 2007; Markovic *et al.* 2014). Moreover, invasive species is a leading cause of species extinctions (Clavero & García-Berthou 2005), thus the presence of non-native freshwater fish in these watersheds most probably conducted to the extinction of native fish species. Indeed, in some cases where non-native species have functionally replaced extinct species, the non-native species have greatly contributed to the native species extinction (Vitule *et al.* 2012).

Second, the spatial scale of the analysis may be unsuitable. Some non-native freshwater fish that are piscivorous (e.g. *Silurus glanis*, *Micropterus salmoides*, and *Sander lucioperca* in Spain) have been introduced in many watersheds to satisfy the demands of recreational fishing (Maceda-Veiga 2013) and their distribution within watersheds is often reduced to big reservoirs and main river channels. Therefore, although the maximum trophic position may have increased at watershed scale due to the introductions of non-native piscivorous fish, the extirpation of native top predators in several parts of the river network within the watersheds may have conducted to the trophic downgrading of these ecosystems at local scale, as occurred in the case study of this PhD thesis. Last, non-native species at high trophic positions may be not functional equivalent to lost top predators. For instance, some prey taxa may not have effective antipredator responses for novel predators due to the lack of predator-prey coevolution (Sih *et al.* 2010; Alvarez, Landeira-Dabarca & Peckarsky 2014). Apex consumers influence ecosystems through complex trophic and non-trophic interactions, thus the consequences of their extinctions and invasions are still unknown and may be unpredictable.

Estes *et al.* (2011) posited that the "loss of apex consumers is arguably humankind's most pervasive influence on the natural world". Like the results of this PhD thesis show, the loss of the top predator may change ecosystem structure and functioning. Consequently, ecosystem restoration actions must be undertaken when top predators are extirpated if managers want to recover previous community structures and ecosystem processes. In our study case, the ecosystem restoration can be simple, it would consist in the reintroduction of *Barbus meridionalis* in the affected streams. But, what is the solution when the top predator is extinct not locally but globally? Extinction eliminates the possibility of reintroduction and leads to the loss of the apex consumer functional role. Therefore, an option is to restore the lost functional role through the introduction of an ecologically similar species in order to fill the niche left vacant by the extinction (i.e. ecological replacement) (Seddon 2010). For instance, *Aldabrachelys* tortoises have been introduced on Mascarene islands to substitute the recently extinct *Cylindraspis* tortoises, restoring their functions of grazing and seed dispersal (Griffiths *et al.* 2010). Lagrue *et al.* (2014) compared the effects of the native noble crayfish (*Astacus astacus*) and the invasive signal crayfish (*Pacifastacus leniusculus*) on macroinvertebrates and leaf breakdown, concluding both crayfish species have similar effects on stream communities. If humans must or not introduce new top predators in ecosystems to replace extinct native species is a philosophical question, but science can help to understand if native and non-native species may perform similar functional roles in ecosystems.

Conclusions

General conclusions

Past fires may have current influence on Mediterranean streams through their effects on the riparian forest and on the aquatic top predator. Although fire is a pulse disturbance, we demonstrated that fire may have long lived effects on the structure and function of Mediterranean streams.

Intermittent streams may be affected by the consequences of top predator extinctions. The effects of the loss of small-bodied fish in intermittent streams are equivalent to local extinctions of larger apex consumers in other ecosystems.

Chapter 1: Long-term consequences of a wildfire for leaf-litter breakdown in a Mediterranean stream

Riparian canopy was reduced by the wildfire, resulting in higher water temperatures and light levels, and reduced leaf-litter inputs in the stream eight years after fire.

Benthic invertebrate communities recovered quickly after the fire, and the abundances of different functional feeding groups, including shredders, were not different between streams affected and unaffected by the fire after >2 postfire years.

Microbially mediated litter decomposition was faster in the stream affected by fire compared to the control, which could be attributed almost entirely to increased water temperatures

Total litter breakdown rates were enhanced by higher shredder abundance in coarse-mesh leaf bags in the fire-affected than in the control stream. Lower leaf-litter inputs in the fire-affected than in the control stream probably led to lower benthic organic matter levels, leading to increased shredder aggregation in leaf packs, thereby accelerating leaf breakdown rates.

Chapter 2: Small but powerful: top predator local extinction affects ecosystem structure and function in an intermittent stream

Top predator extirpation led to 'mesopredator release' in terms of abundance and richness, and also to 'prey release' despite intraguild predation, which contrasts with traditional food web theory. Macroinvertebrate community composition also changed due to *Barbus meridionalis* absence.

Periphyton net primary production was significantly lower in the absence of *B. meridionalis*, confirming a strong trophic cascade effect that modified ecosystem function.

The apex consumer was functionally irreplaceable by the remaining species, despite its small-bodied size and even at low population densities.

Chapter 3: A trait-based approach reveals the feeding selectivity of a small endangered Mediterranean fish

The diet of *Barbus meridionalis* was dominated by chironomids and ephemeropterans. Fish refused most of the potential prey in the stream but positively selected *Cricotopus* spp., *Habrophlebia* sp., and *Stictonectes* sp.

The trait-based analysis showed that 10 of the 13 traits tested significantly influenced food choice: body size, body shape, body flexibility, concealment, locomotion, tendency to drift, diel drift behavior, agility, aggregation tendency, and feeding habits.

Our study showed that prey morphological and behavioral traits may explain prey vulnerability to predation. Therefore, this functional approach can improve our understanding of predator-prey interactions.

Chapter 4: Top predator absence enhances leaf breakdown in a Mediterranean stream

Top predator absence accelerated total leaf breakdown, which was caused by the higher shredder and scraper biomass in coarse-mesh leaf bags in the fishless compared to the control reach. *Barbus meridionalis* changed the whole macroinvertebrate assemblage associated to leaf bags.

Fish absence reduced leaf fungal biomass in both fine- and coarse-mesh bags, but did not decrease microbially mediated leaf breakdown. These results suggested that leaf fungal biomass was bottom-up stimulated through nutrient recycling by the top predator.

References

Abjörnsson, K., Dahl, J., Nyström, P. & Brönmark, C. (2000) Influence of predator and dietary chemical cues on the behaviour and shredding efficiency of *Gammarus pulex*. *Aquatic Ecology*, **34**, 379–387.

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

Allan, D. & Castillo, M.M. (2007) *Stream ecology: structure and function of running waters*, 2nd ed. Springer, Dordrecht.

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

Alvarez, M., Landeira-Dabarca, A. & Peckarsky, B. (2014) Orgin and specificity of predatory fish cues detected by *Baetis* larvae (Ephemeroptera; Insecta). *Animal Behaviour*, **96**, 141–149.

Aparicio, E. & de Sostoa, A. (1999) Pattern of movements of adult *Barbus haasi* in a small Mediterranean stream. *Journal of Fish Biology*, **55**, 1086–1095.

Arkle, R.S. & Pilliod, D.S. (2010) Prescribed fires as ecological surrogates for wildfires: a stream and riparian perspective. *Forest Ecology and Management*, **259**, 893–903.

Atkinson, C.L., Kelly, J.F. & Vaughn, C.C. (2014) Tracing consumer-derived nitrogen in riverine food webs. *Ecosystems*, **17**, 485–496.

Baldy, V. & Gessner, M.O. (1997) Towards a budget of leaf litter decomposition in a first-order woodland stream. *Comptes Rendus de l'Académie des Sciences - Serie III*, **320**, 747–758.

Bärlocher, F. (2005) Leaf mass loss estimated by litter bag technique. *Methods to study litter decomposition: a practical guide* (eds M.A.S. Graça, F. Bärlocher, & M.O. Gessner), pp. 37–42. Springer, Dordrecht.

Baumgärtner, D. & Rothhaupt, K.-O. (2003) Predictive length–dry mass regressions for freshwater invertebrates in a pre-alpine lake littoral. *International Review of Hydrobiology*, **88**, 453–463.

Belmonte-Viudez, M. (2013) *Efectos del barbo de montaña (Barbus meridionalis) sobre la comunidad de macroinvertebrados en un río mediterráneo*. Master thesis, Universidad de Barcelona, Barcelona.

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

Benfield, E.F., Webster, J.R., Tank, J.L. & Hutchens, J.J. (2001) Long-term patterns in leaf breakdown in streams in response to watershed logging. *International Review of Hydrobiology*, **86**, 467–474.

Benke, A.C., Huryn, A.D., Smock, L.A. & Wallace, J.B. (1999) Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *Journal of the North American Benthological Society*, **18**, 308–343.

Benstead, J.P., Cross, W.F., March, J.G., Mcdowell, W.H., Ramírez, A. & Covich, A.P. (2010) Biotic and abiotic controls on the ecosystem significance of consumer excretion in two contrasting tropical streams. *Freshwater Biology*, **55**, 2047–2061.

Benstead, J.P., Rosemond, A.D., Cross, W.F., Wallace, J.B., Eggert, S.L., Suberkropp, K., Gulis, V., Greenwood, J.L. & Tant, C.J. (2009) Nutrient enrichment alters storage and fluxes of detritus in a headwater stream ecosystem. *Ecology*, **90**, 2556–2566.

Bergandi, D. & Blandin, P. (1998) Holism vs. reductionism: do ecosystem ecology and landscape ecology clarify the debate? *Acta Biotheoretica*, **46**, 185–206.

Beschta, R.L. & Ripple, W.J. (2009) Large predators and trophic cascades in terrestrial ecosystems of the western United States. *Biological Conservation*, **142**, 2401–2414.

Betts, E.F. & Jones Jr., J.B. (2009) Impact of wildfire on stream nutrient chemistry and ecosystem metabolism in boreal forest catchments of interior Alaska. *Arctic, Antarctic, and Alpine Research*, **41**, 407–417.

Bixby, R.J., Cooper, S.D., Gresswell, R.E., Brown, L.E., Dahm, C.N. & Dwire, K.A. (2015) Fire effects on aquatic ecosystems: an assessment of the current state of the science. *Freshwater Science*, **34**, 1340–1350.

Boersma, K.S., Bogan, M.T., Henrichs, B.A. & Lytle, D.A. (2014) Top predator removals have consistent effects on large species despite high environmental variability. *Oikos*, **123**, 807–816.

Bogan, M.T., Boersma, K.S. & Lytle, D.A. (2013) Flow intermittency alters longitudinal patterns of invertebrate diversity and assemblage composition in an arid-land stream network. *Freshwater Biology*, **58**, 1016–1028.

Boix, D., Biggs, J., Céréghino, R., Hull, A.P., Kalettka, T. & Oertli, B. (2012) Pond research and management in Europe: “Small is Beautiful.” *Hydrobiologia*, **689**, 1–9.

Bonada, N. & Dolédec, S. (2011) Do mediterranean genera not included in Tachet *et al.* 2002 have mediterranean trait characteristics? *Limnetica*, **30**, 129–142.

Bonada, N., Dolédec, S. & Statzner, B. (2007a) Taxonomic and biological trait differences of stream macroinvertebrate communities between mediterranean and temperate regions: implications for future climatic scenarios. *Global Change Biology*, **13**, 1658–1671.

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

Bonada, N., Rieradevall, M., Prat, N. & Resh, V.H. (2006) Benthic macroinvertebrate assemblages and macrohabitat connectivity in Mediterranean-climate streams of northern California. *Journal of the North American Benthological Society*, **25**, 32–43.

Bond, W.J. & Keeley, J.E. (2005) Fire as a global "herbivore": the ecology and evolution of flammable ecosystems. *TRENDS in Ecology and Evolution*, **20**, 387–394.

Borer, E.T., Seabloom, E., Shurin, J.B., Anderson, K., Blanchette, C.A., Broitman, B., Cooper, S.D. & Halpern, B.S. (2005) What determines the strength of a trophic cascade? *Ecology*, **86**, 528–537.

Borrvall, C. & Ebenman, B. (2006) Early onset of secondary extinctions in ecological communities following the loss of top predators. *Ecology Letters*, **9**, 435–442.

Boukal, D.S. (2014) Trait- and size-based descriptions of trophic links in freshwater food webs: current status and perspectives. *Journal of Limnology*, **73**, 171–185.

Boulêtreau, S., Cucherousset, J., Villéger, S., Masson, R. & Santoul, F. (2011) Colossal aggregations of giant alien freshwater fish as a potential biogeochemical hotspot. *PLoS ONE*, **6**(10): e25732.

Boulton, A.J. & Lake, P.S. (1992a) The ecology of two intermittent streams in Victoria, Australia. III. Temporal changes in faunal composition. *Freshwater Biology*, **27**, 123–138.

Boulton, A.J. & Lake, P.S. (1992b) Benthic organic matter and detritivorous macroinvertebrates in two intermittent streams in south-eastern Australia. *Hydrobiologia*, **241**, 107–118.

Bowman, D.M.J.S., Balch, J., Artaxo, P., Bond, W.J., Cochrane, M.A., D'Antonio, C.M., DeFries, R., Johnston, F.H., Keeley, J.E., Krawchuk, M.A., Kull, C.A., Mack, M., Moritz, M.A., Pyne, S., Roos, C.I., Scott, A.C., Sodhi, N.S. & Swetnam, T.W. (2011) The human dimension of fire regimes on Earth. *Journal of Biogeography*, **38**, 2223–2236.

Boyero, L., Pearson, R.G., Gessner, M.O., Barmuta, L.A., Ferreira, V., Graça, M.A.S., Dudgeon, D., Boulton, A.J., Callisto, M., Chauvet, E., Helson, J.E., Bruder, A., Albariño, R.J., Yule, C.M., Arunachalam, M., Davies, J.N., Figueroa, R., Flecker, A.S., Ramírez, A., Death, R.G., Iwata, T., Mathooko, J.M., Mathuriau, C., Gonçalves, J.F., Moretti, M.S., Jinggut, T., Lamothe, S., M'Erimba, C., Ratnarajah, L., Schindler, M.H., Castela, J., Buria, L.M., Cornejo, A., Villanueva, V.D. & West, D.C. (2011) A global experiment suggests climate warming will not accelerate litter decomposition in streams but might reduce carbon sequestration. *Ecology Letters*, **14**, 289–294.

Boyero, L., Rincón, P.A. & Pearson, R.G. (2008) Effects of a predatory fish on a tropical detritus-based food web. *Ecological Research*, **23**, 649–655.

Braga, R.R., Bornatowski, H. & Vitule, J.R.S. (2012) Feeding ecology of fishes: an overview of worldwide publications. *Reviews in Fish Biology and Fisheries*, **22**, 915–929.

Brashares, J.S., Prugh, L.R., Stoner, C.J. & Epps, C.W. (2010) Ecological and conservation implications of mesopredator release. *Trophic cascades: predators, prey, and the changing dynamics of nature* (eds J. Terborgh & J.A. Estes), pp. 221–240. Island Press, Washington.

Britton, D.L. (1990) Fire and the dynamics of allochthonous detritus in a South African mountain stream. *Freshwater Biology*, **24**, 347–360.

Brown, L.E., Edwards, F.K., Milner, A.M., Woodward, G. & Ledger, M.E. (2011) Food web complexity and allometric scaling relationships in stream mesocosms: implications for experimentation. *Journal of Animal Ecology*, **80**, 884–95.

Brucet, S., Boix, D., Nathansen, L.W., Quintana, X.D., Jensen, E., Balayla, D., Meerhoff, M. & Jeppesen, E. (2012) Effects of temperature, salinity and fish in structuring the macroinvertebrate community in shallow lakes: implications for effects of climate change. *PLoS ONE*, **7**(2): e30877.

Burcher, C.L., Valett, H.M. & Benfield, E.F. (2007) The land-cover cascade: relationships coupling land and water. *Ecology*, **88**, 228–242.

Buria, L.M., Albariño, R.J., Díaz Villanueva, V., Modenutti, B. & Balseiro, E. (2010) Does predation by the introduced rainbow trout cascade down to detritus and algae in a forested small stream in Patagonia? *Hydrobiologia*, **651**, 161–172.

Burton, T.A. (2005) Fish and stream habitat risks from uncharacteristic wildfire: observations from 17 years of fire-related disturbances on the Boise National Forest, Idaho. *Forest Ecology and Management*, **211**, 140–149.

Campeny, R. (2007) *Seguiment de les poblacions d'anfibis del Parc Natural de Sant Llorenç del Munt i l'Obac.* Diputació de Barcelona, Oficina Tècnica de Parcs Naturals, Barcelona.

Capps, K.A. & Flecker, A.S. (2013) Invasive fishes generate biogeochemical hotspots in a nutrient-limited system. *PLoS ONE*, **8**(1): e54093.

Cardillo, M. (2003) Biological determinants of extinction risk: why are smaller species less vulnerable? *Animal Conservation*, **6**, 63–69.

Cardinale, B.J., Srivastava, D.S., Duffy, J.E., Wright, J.P., Downing, A.L., Sankaran, M. & Jouseau, C. (2006) Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature*, **443**, 989–992.

Carlisle, D.M., Wolock, D.M. & Meador, M.R. (2011) Alteration of streamflow magnitudes and potential ecological consequences: a multiregional assessment. *Frontiers in Ecology and the Environment*, **9**, 264–270.

Carpenter, S.R., Kitchell, J. & Hodgson, J. (1985) Cascading trophic interactions and lake productivity. *BioScience*, **35**, 634–639.

Caswell, H. (1978) Predator-mediated coexistence: a nonequilibrium model. *The American Naturalist*, **112**, 127–154.

Cerdà, A. & Doerr, S.H. (2005) Influence of vegetation recovery on soil hydrology and erodibility following fire: an eleven-year investigation. *International Journal of Wildland Fire*, **14**, 423–437.

Chalcraft, D.R. & Resetarits, W.J. (2003) Predator identity and ecological impacts: functional redundancy or functional diversity? *Ecology*, **84**, 2407–2418.

Chase, J.M. (1999) Food web effects of prey size refugia: variable interactions and alternative stable equilibria. *The American Naturalist*, **154**, 559–570.

Cheever, B.M., Webster, J.R., Bilger, E.E. & Thomas, S.A. (2013) The relative importance of exogenous and substrate-derived nitrogen for microbial growth during leaf decomposition. *Ecology*, **94**, 1614–1625.

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

Chung, N. & Suberkropp, K. (2008) Influence of shredder feeding and nutrients on fungal activity and community structure in headwater streams. *Fundamental and Applied Limnology / Archiv für Hydrobiologie*, **173**, 35–46.

Clausen, R. & York, R. (2008) Global biodiversity decline of marine and freshwater fish: a cross-national analysis of economic, demographic, and ecological influences. *Social Science Research*, **37**, 1310–1320.

Clavero, M. & García-Berthou, E. (2005) Invasive species are a leading cause of animal extinctions. *TRENDS in Ecology and Evolution*, **20**, 110–110.

Closs, G.P. (1996) Effects of a predatory fish (*Galaxias olidus*) on the structure of intermittent stream pool communities in southeast Australia. *Australian Journal of Ecology*, **21**, 217–223.

Cohen, J.E. (1977) Ratio of prey to predators in community food webs. *Nature*, **270**, 165–167.

Compte, J., Gascón, S., Quintana, X.D. & Boix, D. (2012) The effects of small fish presence on a species-poor community dominated by omnivores: example of a size-based trophic cascade. *Journal of Experimental Marine Biology and Ecology*, **418-419**, 1–11.

Coombs, J.S. & Melack, J.M. (2013) Initial impacts of a wildfire on hydrology and suspended sediment and nutrient export in California chaparral watersheds. *Hydrological Processes*, **27**, 3842–3851.

Cooper, S.D., Lake, P.S., Sabater, S., Melack, J.M. & Sabo, J.L. (2013) The effects of land use changes on streams and rivers in mediterranean climates. *Hydrobiologia*, **719**, 383–425.

Cooper, S.D., Page, H.M., Wiseman, S.W., Klose, K., Bennett, D.M., Even, T., Sadro, S., Nelson, C.E. & Dudley, T.L. (2015) Physicochemical and biological responses of streams to wildfire severity in riparian zones. *Freshwater Biology*, **60**, 2600–2619.

Cooper, S.D., Walde, S.J. & Peckarsky, B.L. (1990) Prey exchange rates and the impact of predators on prey populations in streams. *Ecology*, **71**, 1503–1514.

Copp, G.H., Spathari, S. & Turmel, M. (2005) Consistency of diel behaviour and interactions of stream fishes and invertebrates during summer. *River Research and Applications*, **21**, 75–90.

Corti, R., Datry, T., Drummond, L. & Larned, S.T. (2011) Natural variation in immersion and emersion affects breakdown and invertebrate colonization of leaf litter in a temporary river. *Aquatic Sciences*, **73**, 537–550.

Costantini, M.L. & Rossi, L. (1998) Competition between two aquatic detritivorous isopods – a laboratory study. *Hydrobiologia*, **368**, 17–27.

Costantini, M.L. & Rossi, L. (2010) Species diversity and decomposition in laboratory aquatic systems: the role of species interactions. *Freshwater Biology*, **55**, 2281–2295.

Costello, M.J., Edwards, J. & Potts, G.W. (1990) The diet of the two-spot goby, *Gobiusculus flavescens* (Pisces). *Journal of the Marine Biological Association of the United Kingdom*, **70**, 329–342.

Cowling, R.M., Ojeda, F., Lamont, B.B., Rundel, P.W. & Lechmere-Oertel, R. (2005) Rainfall reliability, a neglected factor in explaining convergence and divergence of plant traits in fire-prone mediterranean-climate ecosystems. *Global Ecology and Biogeography*, **14**, 509–519.

de Crespin de Billy, V. (2001) *Regime alimentaire de la truite (Salmo trutta L.) en eaux courantes: rôles de l'habitat physique et des traits des macroinvertébrés*. PhD thesis, Université Claude Bernard - Lyon 1, Lyon.

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

Cucherousset, J., Blanchet, S. & Olden, J.D. (2012) Non-native species promote trophic dispersion of food webs. *Frontiers in Ecology and the Environment*, **10**, 406–408.

Danger, M., Cornut, J., Chauvet, E., Chavez, P., Elger, A. & Lecerf, A. (2013) Benthic algae stimulate leaf litter decomposition in detritus-based headwater streams: a case of aquatic priming effect? *Ecology*, **94**, 1604–1613.

Dangles, O. (2002) Functional plasticity of benthic macroinvertebrates: implications for trophic dynamics in acid streams. *Canadian Journal of Fisheries and Aquatic Sciences*, **59**, 1563–1573.

Datry, T., Arscott, D.B. & Sabater, S. (2011) Recent perspectives on temporary river ecology. *Aquatic Sciences*, **73**, 453–457.

Datry, T., Larned, S.T., Fritz, K.M., Bogan, M.T., Wood, P.J., Meyer, E.I. & Santos, A.N. (2014) Broad-scale patterns of invertebrate richness and community composition in temporary rivers: effects of flow intermittence. *Ecography*, **37**, 94–104.

Davey, A.J.H. & Kelly, D.J. (2007) Fish community responses to drying disturbances in an intermittent stream: a landscape perspective. *Freshwater Biology*, **52**, 1719–1733.

DeBano, L.F. (2000) The role of fire and soil heating on water repellency in wildland environments: a review. *Journal of Hydrology*, **231-232**, 195–206.

Diaz, S., Symstad, A.J., Stuart Chapin, F., Wardle, D.A. & Huenneke, L.F. (2003) Functional diversity revealed by removal experiments. *TRENDS in Ecology and Evolution*, **18**, 140–146.

Diehl, S. (1992) Fish predation and benthic community structure: the role of omnivory and habitat complexity. *Ecology*, **73**, 1646–1661.

Diemer, L.A., Mcdowell, W.H., Wymore, A.S. & Prokushkin, A.S. (2015) Nutrient uptake along a fire gradient in boreal streams of Central Siberia. *Freshwater Science*, **34**, 1443–1456.

Dieter, D., Von Schiller, D., García-Roger, E.M., Sánchez-Montoya, M.D.M., Gómez, R., Mora-Gómez, J., Sangiorgio, F., Gelbrecht, J. & Tockner, K. (2011) Preconditioning effects of intermittent stream flow on leaf litter decomposition. *Aquatic Sciences*, **73**, 599–609.

Doadrio, I. (ed). (2001) *Atlas y libro rojo de los peces continentales de España*. Ministerio de Medio Ambiente, Madrid.

Doadrio, I., Perea, S., Garzón-Heydt, P. & González, J.L. (2011) *Ictiofauna continental española. Bases para su seguimiento.* DG Medio Natural y Política Forestal. MARM, Madrid.

Dobson, M. & Hildrew, A. (1992) A test of resource limitation among shredding detritivores in low order streams in southern England. *Journal of Animal Ecology*, **61**, 69–77.

Duffy, J.E. (2002) Biodiversity and ecosystem function: the consumer connection. *Oikos*, **99**, 201–219.

Duffy, J.E., Cardinale, B.J., France, K.E., McIntyre, P.B., Thébault, E. & Loreau, M. (2007) The functional role of biodiversity in ecosystems: incorporating trophic complexity. *Ecology Letters*, **10**, 522–538.

Dufrêne, M. & Legendre, P. (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs*, **67**, 345–366.

Dumont, H.J., Van de Velde, I. & Dumont, S. (1975) The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. *Oecologia*, **19**, 75–97.

Dury, M., Hambuckers, A., Warnant, P., Henrot, A., Favre, E., Ouberdous, M. & François, L. (2011) Responses of European forest ecosystems to 21st century climate: assessing changes in interannual variability and fire intensity. *iForest - Biogeosciences and Forestry*, **4**, 82–99.

Earl, S.R. & Blinn, D.W. (2003) Effects of wildfire ash on water chemistry and biota in South-Western U.S.A. streams. *Freshwater Biology*, **48**, 1015–1030.

Elton, C.S. (1927) *Animal Ecology*. Sidgwick and Jackson, London.

Ernest, S.K. & Brown, J.H. (2001) Delayed compensation for missing keystone species by colonization. *Science*, **292**, 101–104.

Estes, J.A., Terborgh, J., Brashares, J.S., Power, M.E., Berger, J., Bond, W.J., Carpenter, S.R., Essington, T.E., Holt, R.D., Jackson, J.B.C., Marquis, R.J., Oksanen, L., Oksanen, T., Paine, R.T., Pikitch, E.K., Ripple, W.J., Sandin, S.A., Scheffer, M., Schoener, T.W., Shurin, J.B., Sinclair, A.R.E., Soulé, M.E., Virtanen, R. & Wardle, D.A. (2011) Trophic downgrading of planet Earth. *Science*, **333**, 301–306.

European Commission. (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Official Journal of the European Parliament*, **L327**, 1–82.

Farrés-Corell, R. (2005) *Efectes d'un incendi forestal sobre la comunitat de fitobentos a la riera de Gallifa (Vallès Occidental)*. Master thesis, Universitat de Barcelona, Barcelona.

Fausch, K.D., Torgersen, C.E., Baxter, C.V. & Li, H.W. (2002) Landscapes to riverscapes: bridging the gap between research and conservation of stream fishes. *BioScience*, **52**, 483–498.

Ferreira, V. & Chauvet, E. (2011a) Future increase in temperature more than decrease in litter quality can affect microbial litter decomposition in streams. *Oecologia*, **167**, 279–291.

Ferreira, V. & Chauvet, E. (2011b) Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. *Global Change Biology*, **17**, 551–564.

Ferreira, V., Graça, M.A.S., de Lima, J.L.M.P. & Gomes, R. (2006a) Role of physical fragmentation and invertebrate activity in the breakdown rate of leaves. *Archiv für Hydrobiologie*, **165**, 493–513.

Ferreira, V., Gulis, V. & Graça, M.A.S. (2006b) Whole-stream nitrate addition affects litter decomposition and associated fungi but not invertebrates. *Oecologia*, **149**, 718–729.

Finke, D.L. & Denno, R.F. (2005) Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. *Ecology Letters*, **8**, 1299–1306.

Finke, D.L. & Snyder, W.E. (2010) Conserving the benefits of predator biodiversity. *Biological Conservation*, **143**, 2260–2269.

Fioretto, A., Papa, S. & Fuggi, A. (2003) Litter-fall and litter decomposition in a low Mediterranean shrubland. *Biology and Fertility of Soils*, **39**, 37–44.

Foster, D., Swanson, F., Aber, J., Burke, I., Brokaw, N., Tilman, D. & Knapp, A. (2003) The importance of land-use legacies to ecology and conservation. *BioScience*, **53**, 77–88.

Friberg, N. (1997) Benthic invertebrate communities in six Danish forest streams: impact of forest type on structure and function. *Ecography*, **20**, 19–28.

Gagnon, A., Heimpel, G. & Brodeur, J. (2011) The ubiquity of intraguild predation among predatory arthropods. *PLoS ONE*, **6**(1): e28061.

Gallart, F., Prat, N., García-Roger, E.M., Latron, J., Rieradevall, M., Llorens, P., Barberá, G.G., Brito, D., De Girolamo, A.M., Lo Porto, A., Buffagni, A., Erba, S., Neves, R., Nikolaidis, N.P., Perrin, J.L., Querner, E.P., Quinonero, J.M., Tournoud, M.G., Tzoraki, O., Skoulikidis, N., Gamez, R., Gomez, R. & Froebrich, J. (2012) A novel approach to analysing the regimes of temporary streams in relation to their controls on the composition and structure of aquatic biota. *Hydrology and Earth System Sciences*, **16**, 3165–3182.

Gama, M., Gonçalves, A.L., Ferreira, V., Graça, M.A.S. & Canhoto, C. (2007) Decomposition of fire exposed Eucalyptus leaves in a Portuguese lowland stream. *International Review of Hydrobiology*, **92**, 229–241.

García-Berthou, E. (1994) *Ecologia alimentària de la comunitat de peixos de l'estany de Banyoles*. PhD thesis, Universitat de Girona, Girona.

García-Roger, E.M., Sánchez-Montoya, M.D.M., Gómez, R., Suárez, M.L., Vidal-Abarca, M.R., Latron, J., Rieradevall, M. & Prat, N. (2011) Do seasonal changes in habitat features influence aquatic macroinvertebrate assemblages in perennial versus temporary Mediterranean streams? *Aquatic Sciences*, **73**, 567–579.

Gasith, A. & Resh, V. (1999) Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. *Annual Review of Ecology and Systematics*, **30**, 51–81.

Gelwick, F.P., Stock, M.S. & Matthews, W.J. (1997) Effects of fish, water depth, and predation risk on patch dynamics in a north-temperate river ecosystem. *Oikos*, **80**, 382–398.

Gessner, M.O. (2005) Ergosterol as a measure of fungal biomass. *Methods to study litter decomposition: a practical guide* (eds M.A.S. Graça, F. Bärlocher, & M.O. Gessner), pp. 171–176. Springer, Dordrecht.

Gessner, M.O. & Chauvet, E. (1993) Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. *Applied and Environmental Microbiology*, **59**, 502–507.

Gessner, M.O., Chauvet, E. & Dobson, M. (1999) A perspective on leaf litter breakdown in streams. *Oikos*, **85**, 377–384.

Gessner, M.O., Robinson, C.T. & Ward, J.V. (1998) Leaf breakdown in streams of an alpine glacial floodplain: dynamics of fungi and nutrients. *Journal of the North American Benthological Society*, **17**, 403–419.

Gido, K.B., Bertrand, K.N., Murdock, J.N., Dodds, W.K. & 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.

Gilliam, J., Fraser, D.F. & Sabat, A.M. (1989) Strong effects of foraging minnows on a stream benthic invertebrate community. *Ecology*, **70**, 445–452.

Godwin, C.M. & Carrick, H.J. (2008) Spatio-temporal variation of periphyton biomass and accumulation in a temperate spring-fed stream. *Aquatic Ecology*, **42**, 583–595.

González, E. (2012) Seasonal patterns of litterfall in the floodplain forest of a large Mediterranean river. *International Review of Hydrobiology*, **31**, 173–186.

González, J.M. & Graça, M.A.S. (2005) Influence of detritus on the structure of the invertebrate community in a small Portuguese stream. *International Review of Hydrobiology*, **90**, 534–545.

Graça, M.A.S. (2001) The role of invertebrates on leaf litter decomposition in streams – a review. *International Review of Hydrobiology*, **86**, 383–394.

Graça, M.A.S. & Canhoto, C. (2006) Leaf litter processing in low order streams. *Limnetica*, **25**, 1–10.

Graça, M.A.S. & Zimmer, M. (2005) Leaf toughness. *Methods to study litter decomposition: a practical guide* (eds M.A.S. Graça, F. Bärlocher, & M.O. Gessner), pp. 109–113. Springer, Dordrecht.

Green, S.J. & Côté, I.M. (2014) Trait-based diet selection: prey behaviour and morphology predict vulnerability to predation in reef fish communities. *Journal of Animal Ecology*, **83**, 1451–1460.

Greig, H.S. & McIntosh, A.R. (2006) Indirect effects of predatory trout on organic matter processing in detritus-based stream food webs. *Oikos*, **112**, 31–40.

Gresswell, R.E. (1999) Fire and aquatic ecosystems in forested biomes of North America. *Transactions of the American fisheries society*, **128**, 193–221.

Griffiths, C.J., Jones, C.G., Hansen, D.M., Puttoo, M., Tatayah, R.V., Müller, C.B. & Harris, S. (2010) The use of extant non-indigenous tortoises as a restoration tool to replace extinct ecosystem engineers. *Restoration Ecology*, **18**, 1–7.

Guinart, D. (2007) Restoration of the burnt ecosystem in the eastern sector of the Sant Llorenç del Munt i l'Obac nature reserve. *VI Trobada d'Estudiosos de Sant Llorenç del Munt i l'Obac* (eds J. Hernández, J. Melero, & J. Grau), pp. 37–44. Diputació de Barcelona, Barcelona.

Gulis, V. & Suberkropp, K. (2003) Leaf litter decompositioin and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater Biology*, **48**, 123–134.

Gurevitch, J., Morrison, J. & Hedges, L. (2000) The interaction between competition and predation: a meta-analysis of field experiments. *The American Naturalist*, **155**, 435–453.

Hairston, N.G., Smith, F. & Slobodkin, L. (1960) Community structure, population control, and competition. *The American Naturalist*, **94**, 421–425.

Hall, S.J. & Lombardozzi, D. (2008) Short-term effects of wildfire on montane stream ecosystems in the Southern Rocky Mountains: one and two years post-burn. *Western North American Naturalist*, **68**, 453–462.

Harding, J.S., Benfield, E.F., Bolstad, P.V., Helfman, G.S. & Jones III, E.B.D. (1998) Stream biodiversity: the ghost of land use past. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 14843–14847.

Heithaus, M.R., Frid, A., Wirsing, A.J. & Worm, B. (2008) Predicting ecological consequences of marine top predator declines. *TRENDS in Ecology and Evolution*, **23**, 202–210.

Henke, S.E. & Bryant, F.C. (1999) Effects of coyote removal on the faunal community in western Texas. *The Journal of Wildlife Management*, **63**, 1066–1081.

Hladyz, S., Watkins, S.C., Whitworth, K.L. & Baldwin, D.S. (2011) Flows and hypoxic blackwater events in managed ephemeral river channels. *Journal of Hydrology*, **401**, 117–125.

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

Holt, R.D. & Huxel, G.R. (2007) Alternative prey and the dynamics of intraguild predation: theoretical perspectives. *Ecology*, **88**, 2706–2712.

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

Hynes, H.B.N. (1975) The stream and its valley. *Verhandlungen des Internationalen Verein Limnologie*, **19**, 1–15.

Inbar, M., Tamir, M. & Wittenberg, L. (1998) Runoff and erosion processes after a forest fire in Mount Carmel, a Mediterranean area. *Geomorphology*, **24**, 17–33.

Ings, T.C., Montoya, J.M., Bascompte, J., Blüthgen, N., Brown, L.E., Dormann, C.F., Edwards, F., Figueroa, D., Jacob, U., Jones, J.I., Lauridsen, R.B., Ledger, M.E., Lewis, H.M., Olesen, J.M., Van Veen, F.J.F., Warren, P.H. & Woodward, G. (2009) Ecological networks - Beyond food webs. *Journal of Animal Ecology*, **78**, 253–269.

IPCC. (2014) *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* (eds C.B. Field, V.R. Barros, D.J. Dokken, K.J. Mach, M.D. Mastrandrea, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea, & L.L. White). Cambridge University Press, Cambridge and New York.

Irons III, J.G., Oswood, M.W., Stout, R.J. & Pringle, C.M. (1994) Latitudinal patterns in leaf litter breakdown: is temperature really important? *Freshwater Biology*, **32**, 401–411.

Ives, A.R., Cardinale, B.J. & Snyder, W.E. (2004) A synthesis of subdisciplines: predator-prey interactions, and biodiversity and ecosystem functioning. *Ecology Letters*, **8**, 102–116.

Jabiol, J., McKie, B.G. & Bruder, A. (2013) Trophic complexity enhances ecosystem functioning in an aquatic detritus-based model system. *Journal of Animal Ecology*, **82**, 1042–1051.

Jackson, B.K., Sullivan, S.M.P. & Malison, R.L. (2012) Wildfire severity mediates fluxes of plant material and terrestrial invertebrates to mountain streams. *Forest Ecology and Management*, **278**, 27–34.

Jacobs, J. (1974) Quantitative measurement of food selection. *Oecologia*, **14**, 413–417.

Jeffrey, S.W. & Humphrey, G.F. (1975) New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochemical Physiology Pflanz*, **167**, 191–194.

Jeffries, M.J. (2002) Evidence for individualistic species assembly creating convergent predator:prey ratios among pond invertebrate communities. *Journal of Animal Ecology*, **71**, 173–184.

Jenkins, M. (2003) Prospects for biodiversity. *Science*, **302**, 1175–1177.

Johannesen, A., Dunn, A.M. & Morrell, L.J. (2014) Prey aggregation is an effective olfactory predator avoidance strategy. *PeerJ*, **2**: e408.

Johnson, B.R. & Wallace, J.B. (2005) Bottom-up limitation of a stream salamander in a detritus-based food web. *Canadian Journal of Fisheries and Aquatic Sciences*, **62**, 301–311.

Kéfi, S., Berlow, E.L., Wieters, E.A., Navarrete, S.A., Petchey, O.L., Wood, S.A., Boit, A., Joppa, L.N., Lafferty, K.D., Williams, R.J., Martinez, N.D., Menge, B.A., Blanchette, C.A., Iles, A.C. & Brose, U. (2012) More than a meal… integrating non-feeding interactions into food webs. *Ecology Letters*, **15**, 291–300.

Klecka, J. (2014) Modelling size structured food webs using a modified niche model with two predator traits. *PLoS ONE*, **9**(8): e99355.

Klecka, J. & Boukal, D.S. (2013) Foraging and vulnerability traits modify predator-prey body mass allometry: freshwater macroinvertebrates as a case study. *Journal of Animal Ecology*, **82**, 1031–1041.

Klose, K., Cooper, S.D. & Bennett, D.M. (2015) Effects of wildfire on stream algal abundance, community structure, and nutrient limitation. *Freshwater Science*, **34**, 1494–1509.

Knight, T.M., McCoy, M.W., Chase, J.M., McCoy, K.A. & Holt, R.D. (2005) Trophic cascades across ecosystems. *Nature*, **437**, 880–883.

Koetsier, P., Krause, T.R.B. & Tuckett, Q.M. (2010) Present effects of past wildfires on leaf litter breakdown in stream ecosystems. *Western North American Naturalist*, **70**, 164–174.

Konishi, M., Nakano, S. & Iwata, T. (2001) Trophic cascading effects of predatory fish on leaf litter. *Ecological Research*, **16**, 415–422.

Kotliar, N.B., Baker, B.W., Whicker, A.D. & Plumb, G. (1999) A critical review of assumptions about the prairie dog as a keystone species. *Environmental Management*, **24**, 177–192.

Lafferty, K.D. (2004) Fishing for lobsters indirectly increases epidemics in sea urchins. *Ecological Applications*, **14**, 1566–1573.

Lagrue, C., Besson, A.A. & Lecerf, A. (2015) Interspecific differences in antipredator strategies determine the strength of non-consumptive predator effects on stream detritivores. *Oikos*, **124**, 1589-1596.

Lagrue, C., Kominoski, J.S., Danger, M., Baudoin, J.-M., Lamothe, S., Lambrigot, D. & Lecerf, A. (2011) Experimental shading alters leaf litter breakdown in streams of contrasting riparian canopy cover. *Freshwater Biology*, **56**, 2059–2069.

Lagrue, C., Podgorniak, T., Lecerf, A. & Bollache, L. (2014) An invasive species may be better than none: invasive signal and native noble crayfish have similar community effects. *Freshwater Biology*, **59**, 1982–1995.

Lamberti, G. & Steinman, A. (1993) Research in artificial streams: applications, uses, and abuses. *Journal of the North American Benthological Society*, **12**, 313–384.

Lancaster, J., Hildrew, A. & Townsend, C.R. (1991) Invertebrate predation on patchy and mobile prey in streams. *Journal of Animal Ecology*, **60**, 625–641.

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

Lecerf, A. & Richardson, J.S. (2010) Litter decomposition can detect effects of high and moderate levels of forest disturbance on stream condition. *Forest Ecology and Management*, **259**, 2433–2443.

Lecerf, A. & Richardson, J.S. (2011) Assessing the functional importance of large-bodied invertebrates in experimental headwater streams. *Oikos*, **120**, 950–960.

Lee, P.Y. & Suen, J.P. (2014) Dependency and independency among fish density and electivity indices in a stream fish assemblage. *Environmental Biology of Fishes*, **97**, 111–119.

Leigh, C., Boulton, A.J., Courtwright, J.L., Fritz, K., May, C.L., Walker, R.H. & Datry, T. (in press) Ecological research and management of intermittent rivers: an historical review and future directions. *Freshwater Biology*.

Linklater, W. (1995) Breakdown and detritivore colonisation of leaves in three New Zealand streams. *Hydrobiologia*, **306**, 241–250.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U., Huston, M.A, Raffaelli, D., Schmid, B., Tilman, D. & Wardle, D.A. (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science*, **294**, 804–808.

Love, J.W., Taylor, C.M. & Warren, M.L. (2005) Predator density and dissolved oxygen affect body condition of *Stenonema tripunctatum* (Ephemeroptera, Heptageniidae) from intermittent streams. *Hydrobiologia*, **543**, 113–118.

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

Maceda-Veiga, A. (2013) Towards the conservation of freshwater fish: Iberian Rivers as an example of threats and management practices. *Reviews in Fish Biology and Fisheries*, **23**, 1–22.

Machovina, B., Feeley, K.J. & Ripple, W.J. (2015) Biodiversity conservation: the key is reducing meat consumption. *Science of The Total Environment*, **536**, 419–431.

Magalhaes, M.F. (1992) Feeding ecology of the Iberian cyprinid *Barbus bocagei* Steindachner, 1865 in a lowland river. *Journal of Fish Biology*, **40**, 123–133.

Majdi, N., Traunspurger, W., Richardson, J.S. & Lecerf, A. (2015) Small stonefly predators affect microbenthic and meiobenthic communities in stream leaf packs. *Freshwater Biology*, **60**, 1930–1943.

Malmqvist, B. (1993) Interactions in stream leaf packs - effects of a stonefly predator on detritivores and organic-matter processing. *Oikos*, **66**, 454–462.

Mancinelli, G., Costantini, M.L. & Rossi, L. (2007) Top-down control of reed detritus processing in a lake littoral zone: experimental evidence of a seasonal compensation between fish and invertebrate predation. *International Review of Hydrobiology*, **92**, 117–134.

Markovic, D., Carrizo, S., Freyhof, J., Cid, N., Lengyel, S., Scholz, M., Kasperdius, H. & Darwall, W. (2014) Europe's freshwater biodiversity under climate change: distribution shifts and conservation needs. *Diversity and Distributions*, **20**, 1097–1107.

Marshall, K.N. & Essington, T.E. (2011) Energetic conditions promoting top-down control of prey by predators. *PLoS ONE*, **6**(12): e29723.

Martínez, A., Larrañaga, A., Pérez, J., Descals, E. & Pozo, J. (2014) Temperature affects leaf litter decomposition in low-order forest streams: field and microcosm approaches. *FEMS Microbiology Ecology*, **87**, 257–67.

Martinussen, P.A., Robertsen, G. & Einum, S. (2011) Density-dependent diet composition of juvenile Atlantic salmon (*Salmo salar*). *Ecology of Freshwater Fish*, **20**, 384–392.

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

Mayor, A.G., Bautista, S., Llovet, J. & Bellot, J. (2007) Post-fire hydrological and erosional responses of a Mediterranean landscpe: seven years of catchment-scale dynamics. *Catena*, **71**, 68–75.

Mazor, R.D., Stein, E.D., Ode, P.R. & Schiff, K. (2014) Integrating intermittent streams into watershed assessments: applicability of an index of biotic integrity. *Freshwater Science*, **33**, 459–474.

McArthur, J.V., Barnes, J.R., Hansen, B.J. & Leff, L.G. (1988) Seasonal dynamics of leaf litter breakdown in a Utah alpine stream. *Journal of the North American Benthological Society*, **7**, 44–50.

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

McIntyre, P.B., Jones, L.E., Flecker, A.S. & Vanni, M.J. (2007) Fish extinctions alter nutrient recycling in tropical freshwaters. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 4461–4466.

Meerhoff, M., Iglesias, C., de Mello, F.T., Clemente, J.M., Jensen, E., Lauridsen, T.L. & Jeppesen, E. (2007) Effects of habitat complexity on community structure and predator avoidance behaviour of littoral zooplankton in temperate versus subtropical shallow lakes. *Freshwater Biology*, **52**, 1009–1021.

Meixner, T., Fenn, M.E., Wohlgemuth, P., Oxford, M. & Riggan, P. (2006) N saturation symptoms in chaparral catchments are not reversed by prescribed fire. *Environmental Science & Technology*, **40**, 2887–2894.

Mellon, C.D., Wipfli, M.S. & Li, J.L. (2008) Effects of forest fire on headwater stream macroinvertebrate communities in eastern Washington, U.S.A. *Freshwater Biology*, **53**, 2331–2343.

Menéndez, M., Hernández, O. & Comín, F.A. (2003) Seasonal comparisons of leaf processing rates in two Mediterranean rivers with different nutrient availability. *Hydrobiologia*, **495**, 159–169.

Merritt, R. & Cummins, K.W. (1996) *An introduction to the aquatic insects of North America*, 3rd ed. Kendal/Hunt, Duduque.

Meyer, E. (1989) The relationship between body length parameters and dry mass in running water invertebrates. *Archiv für Hydrobiologie*, **117**, 191–203.

Meyer, J.L., Strayer, D.L., Wallace, J.B., Eggert, S.L., Helfman, G.S. & Leonard, N.E. (2007) The contribution of headwater streams to biodiversity in river networks. *Journal of the American Water Resources Association*, **43**, 86–103.

Mihuc, T.B. & Minshall, G.W. (1995) Trophic generalists vs. trophic specialists: implications for food web dynamics in post-fire streams. *Ecology*, **76**, 2361–2372.

Mihuc, T.B. & Minshall, G.W. (2005) The trophic basis of reference and post-fire stream food webs 10 years after wildfire in Yellowstone National Park. *Aquatic Sciences*, **67**, 541–548.

Minshall, G.W. (2003) Responses of stream benthic macroinvertebrates to fire. *Forest Ecology and Management*, **178**, 155–161.

Minshall, G.W., Petersen, R.C., Cummins, K.W., Bott, T.L., Sedell, J.R., Cushing, C.E. & Vannote, R.L. (1983) Interbiome comparison of stream ecosystem dynamics. *Ecological Monographs*, **53**, 1–25.

Minshall, G.W., Robinson, C.T. & Lawrence, D.E. (1997) Postfire responses of lotic ecosystems in Yellowstone National Park, U.S.A. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 2509–2525.

Minshall, G.W., Royer, T.V. & Robinson, C.T. (2001) Response of the Cache Creek macroinvertebrates during the first 10 years following disturbance by the 1988 Yellowstone wildfires. *Canadian Journal of Fisheries and Aquatic Sciences*, **58**, 1077–1088.

Miserendino, M. (2001) Length-mass relationships for macroinvertebrates in freshwater environments of Patagonia (Argentina). *Ecología Austral*, **11**, 3–8.

Miyashita, T. & Niwa, S. (2006) A test for top-down cascade in a detritus-based food web by litter-dwelling web spiders. *Ecological Research*, **21**, 611–615.

Molles, M.C. (1982) Trichopteran communities of streams associated with aspen and conifer forests: long-term structural change. *Ecology*, **63**, 1–6.

Moore, J.W., Schindler, D.E., Carter, J.L., Fox, J., Griffiths, J. & Holtgrieve, G.W. (2007) Biotic control of stream fluxes: spawning salmon drive nutrient and matter export. *Ecology*, **88**, 1278–1291.

Mulholland, P.J., Helton, A.M., Poole, G.C., Hall, R.O., Hamilton, S.K., Peterson, B.J., Tank, J.L., Ashkenas, L.R., Cooper, L.W., Dahm, C.N., Dodds, W.K., Findlay, S.E.G., Gregory, S.V., Grimm, N.B., Johnson, S.L., McDowell, W.H., Meyer, J.L., Valett, H.M., Webster, J.R., Arango, C.P., Beaulieu, J.J., Bernot, M.J., Burgin, A.J., Crenshaw, C.L., Johnson, L.T., Niederlehner, B.R., O'Brien, J.M., Potter, J.D., Sheibley, R.W., Sobota, D.J. & Thomas, S.M. (2008) Stream denitrification across biomes and its response to anthropogenic nitrate loading. *Nature*, **452**, 202–205.

Munné, A., Prat, N., Solà, C., Bonada, N. & Rieradevall, M. (2003) A simple field method for assessing the ecological quality of riparian habitat in rivers and streams: QBR index. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **13**, 147–163.

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

Nikolaidis, N.P., Demetropoulou, L., Froebrich, J., Jacobs, C., Gallart, F., Prat, N., Lo Porto, A., Campana, C., Papadoulakis, V., Skoulikidis, N., Davy, T., Bidoglio, G., Bouraoui, F., Kirkby, M., Tournoud, M.G., Polesello, S., Barberá, G.G., Cooper, D., Gomez, R., Sánchez-Montoya, M.D.M., Latron, J., De Girolamo, A.M. & Perrin, J.L. (2013) Towards sustainable management of Mediterranean river basins: policy recommendations on management aspects of temporary streams. *Water Policy*, **15**, 830–849.

Nordgren, A. (2012) Meat and global warming: impact models, mitigation approaches and ethical aspects. *Environmental Values*, **21**, 437–457.

Obernborfer, R.Y., Mcarthur, J.V., Barnes, J.R., Dixon, J. & Oberndorfer, R.Y. (1984) The effect of invertebrate predators on leaf litter processing in an alpine stream. *Ecology*, **65**, 1325–1331.

Ohta, T., Miyake, Y. & Hiura, T. (2011) Light intensity regulates growth and reproduction of a snail grazer (*Gyraulus chinensis*) through changes in the quality and biomass of stream periphyton. *Freshwater Biology*, **56**, 2260–2271.

Olden, J.D., Hogan, Z.S. & Zanden, M.J. Vander. (2007) Small fish, big fish, red fish, blue fish: size-biased extinction risk of the world's freshwater and marine fishes. *Global Ecology and Biogeography*, **16**, 694–701.

Oliver, A.A., Bogan, M.T., Herbst, D.B. & Dahlgren, R.A. (2012) Short-term changes in-stream macroinvertebrate communities following a severe fire in the Lake Tahoe basin, California. *Hydrobiologia*, **694**, 117–130.

Ordiz, A., Bischof, R. & Swenson, J.E. (2013) Saving large carnivores, but losing the apex predator? *Biological Conservation*, **168**, 128–133.

Oscoz, J., Leunda, P.M., Campos, F., Escala, M.C. & Miranda, R. (2005) Diet of 0+ brown trout (*Salmo trutta* L., 1758) from the river Erro (Navarra, north of Spain). *Limnetica*, **24**, 319–326.

Paine, R. (1969) A note on trophic complexity and community stability. *The American Naturalist*, **103**, 91–93.

Pancotto, V.A., Sala, O.E., Cabello, M., López, N.I., Robson, T.M., Ballaré, C.L., Caldwell, M.M. & Scopel, A.L. (2003) Solar UV-B decreases decomposition in herbaceous plant litter in Tierra del Fuego, Argentina: potential role of an altered decomposer community. *Global Change Biology*, **9**, 1465–1474.

Pastor, A., Compson, Z.G., Dijkstra, P., Riera, J.L., Martí, E., Sabater, F., Hungate, B.A. & Marks, J.C. (2014) Stream carbon and nitrogen supplements during leaf litter decomposition: contrasting patterns for two foundation species. *Oecologia*, **176**, 1111–1121.

Pausas, J.G., Llovet, J., Anselm, R. & Vallejo, R. (2008) Are wildfires a disaster in the Mediterranean basin ? – a review. *International Journal of Wildland Fire*, **17**, 713–723.

Petchey, O.L., Beckerman, A., Riede, J.O. & Warren, P.H. (2008) Size, foraging, and food web structure. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 4191–4196.

Petchey, O.L., Downing, A.L., Mittelbach, G.G., Persson, L., Steiner, C.F., Warren, P.H. & Woodward, G. (2004) Species loss and the structure and functioning of multitrophic aquatic systems. *Oikos*, **104**, 467–478.

Piggott, J.J., Lange, K., Townsend, C.R. & Matthaei, C.D. (2012) Multiple stressors in agricultural streams: a mesocosm study of interactions among raised water temperature, sediment addition and nutrient enrichment. *PLoS ONE*, **7**(11): e49873.

Pilliod, D.S., Bury, R.B., Hyde, E.J., Pearl, C.A. & Corn, P.S. (2003) Fire and amphibians in North America. *Forest Ecology and Management*, **178**, 163–181.

Polis, G.A., Myers, C.A. & Holt, R.D. (1989) The ecology and evolution of intraguild predation: potential competitors that eat each other. *Annual Review of Ecology and Systematics*, **20**, 297–330.

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

Pozo, J., González, E. & Díez, J.R. (1997) Inputs of particulate organic matter to streams with different riparian vegetation. *Journal of the North American Benthological Society*, **16**, 602–611.

Prat, N., Gallart, F., Von Schiller, D., Polesello, S., García-Roger, E.M., Latron, J., Rieradevall, M., Llorens, P., Barberá, G.G., Brito, D., De Girolamo, A.M., Dieter, D., lo Porto, A., Buffagni, A., Erba, S., Nikolaidis, N.P., Querner, E.P., Tournoud, M.G., Tzoraki, O., Skoulikidis, N., Gómez, R., Sánchez-Montoya, M.M., Tockner, K. &

Froebrich, J. (2014) The Mirage toolbox: an integrated assessment tool for temporary streams. *River Research and Applications*, **30**, 1318–1334.

Prat, N., Puig, M.A. & González, G. (1983) *Predicció i control de la qualitat de les aigües dels rius Besòs i Llobregat, II. El poblament faunístic i la seva relació amb la qualitat de les aigües*. Diputació de Barcelona, Servei del Medi Ambient, Barcelona.

Prat, N., Puig, M.A., González, G. & Tort, M.J. (1982) *Predicció i control de la qualitat de les aigües dels rius Besòs i Llobregat, I. Els factors físics i químics del medi*. Diputació de Barcelona, Servei del Medi Ambient, Barcelona.

Preisser, E.L., Bolnick, D.I. & Benard, M.F. (2005) Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology*, **86**, 501–509.

Pringle, C.M., Naiman, J.R., Bretschko, G., Karr, J.R., Oswood, M.W., Webster, J.R., Welcomme, R.L. & Winterbourn, M.J. (1988) Patch dynamics in lotic systems: the strean as a mosaic. *Journal of the North American Benthological Society*, **7**, 503–524.

Prugh, L.R., Stoner, C.J., Epps, C.W., Bean, W.T., Ripple, W.J., Laliberte, A.S. & Brashares, J.S. (2009) The rise of the mesopredator. *BioScience*, **59**, 779–791.

Puntí, T., Rieradevall, M. & Prat, N. (2009) Environmental factors, spatial variation, and specific requirements of Chironomidae in Mediterranean reference streams. *Journal of the North American Benthological Society*, **28**, 247–265.

Purvis, A., Gittleman, J.L., Cowlishaw, G. & Mace, G.M. (2000) Predicting extinction risk in declining species. *Proceedings of the Royal Society B: Biological Sciences*, **267**, 1947–1952.

Pyke, G. (1984) Optimal foraging theory: a critical review. *Annual Review of Ecology and Systematics*, **15**, 523–575.

R Core Team. (2012) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

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

Ramsay, P.M., Rundle, S.D., Attrill, M.J., Uttley, M.G., Williams, P.R., Elsmere, P.S. & Abada, A. (1997) A rapid method for estimating biomass size spectra of benthic metazoan communities. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 1716–1724.

Real, M., Rieradevall, M. & Prat, N. (1989) Limnologia de les rieres del Massís de Sant Llorenç del Munt i l'Obac. *I Trobada d'estudiosos de Sant Llorenç del Munt i Serra de l'Obac* pp. 79–81. Diputació de Barcelona, Barcelona.

Reice, S. (1991) Effects of detritus loading and fish predation on leafpack breakdown and benthic macroinvertebrates in a woodland stream. *Journal of the North American Benthological Society*, **10**, 42–56.

Reich, P., McMaster, D., Bond, N., Metzeling, L. & Lake, P.S. (2010) Examining the ecological consequences of restoring flow intermittency to artificially perennial lowland streams: patterns and predictions from the Broken—Boosey creek system in Northern Victoria, Australia. *River Research and Applications*, **26**, 529–545.

Rezende, R.D.S., Leite, G.F.M., De-Lima, A.K.S., Silva Filho, L.A.B., Chaves, C.V.C., Prette, A.C.H., Freitas, J.S. & Gonçalves Júnior, J.F. (2015) Effects of density and predation risk on leaf litter processing by *Phylloicus* sp. *Austral Ecology*, **40**, 693–700.

Rhoades, C.C., Entwistle, D. & Butler, D. (2011) The influence of wildfire extent and severity on streamwater chemistry, sediment and temperature following the Hayman Fire, Colorado. *International Journal of Wildland Fire*, **20**, 430–442.

Richardson, J.S. (1991) Seasonal food limitation of detritivores in a montane stream: an experimental test. *Ecology*, **72**, 873–887.

Rieradevall, M., Bonada, N. & Prat, N. (1999) Community structure and water quality in the Mediterranean streams of a natural park (St. Llorenç del Munt, NE Spain). *Limnetica*, **17**, 45–56.

Rincón, P.A. & Lobón-Cerviá, J. (1999) Prey-size selection by brown trout (*Salmo trutta* L.) in a stream in northern Spain. *Canadian Journal of Zoology*, **77**, 755–765.

Rinne, J.N. (1996) Short-term effects of wildfire on fishes and aquatic macroinvertebrates in the Southwestern United States. *North American Journal of Fisheries Management*, **16**, 653–658.

Ripple, W.J., Estes, J.A, Beschta, R.L., Wilmers, C.C., Ritchie, E.G., Hebblewhite, M., Berger, J., Elmhagen, B., Letnic, M., Nelson, M.P., Schmitz, O.J., Smith, D.W., Wallach, A.D. & Wirsing, A.J. (2014) Status and ecological effects of the world's largest carnivores. *Science*, **343**.

Ripple, W.J., Wirsing, A.J., Wilmers, C.C. & Letnic, M. (2013) Widespread mesopredator effects after wolf extirpation. *Biological Conservation*, **160**, 70–79.

Ritchie, E.G. & Johnson, C.N. (2009) Predator interactions, mesopredator release and biodiversity conservation. *Ecology Letters*, **12**, 982–998.

Robinson, C.T. & Gessner, M.O. (2000) Nutrient addition accelerates leaf breakdown in an alpine springbrook. *Oecologia*, **122**, 258–263.

Robinson, C.T., Uehlinger, U. & Minshall, G.W. (2005) Functional characteristics of wilderness streams twenty years following wildfire. *Western North American Naturalist*, **65**, 1–10.

Roby, K.B. & Azuma, D.L. (1995) Changes in a reach of a northern California stream following wildfire. *Environmental Management*, **19**, 591–600.

Rocha, L.G., Medeiros, E.S.F. & Andrade, H.T.A. (2012) Influence of flow variability on macroinvertebrate assemblages in an intermittent stream of semi-arid Brazil. *Journal of Arid Environments*, **85**, 33–40.

Rodríguez-Lozano, P. (2010) *Fuego y sequía: efectos sobre la estructura y los rasgos biológicos de las comunidades de macroinvertebrados fluviales*. Master thesis, Universidad de Barcelona, Barcelona.

Rodríguez-Lozano, P., Rieradevall, M., Rau, M.A. & Prat, N. (2015a) Long-term consequences of a wildfire for leaf-litter breakdown in a Mediterranean stream. *Freshwater Science*, **34**, 1482–1493.

Rodríguez-Lozano, P., Verkaik, I., Rieradevall, M. & Prat, N. (2015b) Small but powerful: top predator local extinction affects ecosystem structure and function in an intermittent stream. *PLoS ONE*, **10**(2): e0117630.

Rohr, R.P., Scherer, H., Kehrli, P., Mazza, C. & Bersier, L.-F. (2010) Modeling food webs: exploring unexplained structure using latent traits. *The American Naturalist*, **176**, 170–177.

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.

Rosenheim, J.A., Kaya, H., Ehler, L., Marois, J.M. & Jaffee, B.A. (1995) Intraguild predation among biological-control agents: theory and evidence. *Biological control*, **5**, 303–335.

Rowe, L. & Richardson, J.S. (2001) Community responses to experimental food depletion: resource tracking by stream invertebrates. *Oecologia*, **129**, 473–480.

Rudolf, V. (2007) The interaction of cannibalism and omnivory: consequences for community dynamics. *Ecology*, **88**, 2697–2705.

Ruetz, C.R., Newman, R. & Vondracek, B. (2002) Top-down control in a detritus-based food web: fish, shredders, and leaf breakdown. *Oecologia*, **132**, 307–315.

Saddlier, S., Koehn, J.D. & Hammer, M.P. (2013) Let's not forget the small fishes - conservation of two threatened species of pygmy perch in south-eastern Australia. *Marine and Freshwater Research*, **64**, 874–886.

Sánchez-Hernández, J. & Cobo, F. (2015) Adaptive flexibility in the feeding behaviour of brown trout: optimal prey size. *Zoological Studies*, **54**, 1–9.

Sánchez-Hernández, J., Servia, M.J., Vieira-Lanero, R. & Cobo, F. (2012) Aplicación de los rasgos ecológicos ("traits") de las presas para el estudio del comportamiento alimentario en peces bentófagos: el ejemplo del espinoso (*Gasterosteus gymnurus* Cuvier, 1829). *Limnetica*, **31**, 59–76.

Sánchez-Hernández, J., Vieira-Lanero, R., Servia, M.J. & Cobo, F. (2011) Feeding habits of four sympatric fish species in the Iberian Peninsula: keys to understanding coexistence using prey traits. *Hydrobiologia*, **667**, 119–132.

Sandler, R. (2010) The value of species and the ethical foundations of assisted colonization. *Conservation Biology*, **24**, 424–31.

Säterberg, T., Sellman, S. & Ebenman, B. (2013) High frequency of functional extinctions in ecological networks. *Nature*, **499**, 468–471.

Sax, D.F. & Gaines, S.D. (2003) Species diversity: from global decreases to local increases. *TRENDS in Ecology and Evolution*, **18**, 561–566.

von Schiller, D., Acuña, V., Graeber, D., Martí, E., Ribot, M., Sabater, S., Timoner, X. & Tockner, K. (2011) Contraction, fragmentation and expansion dynamics determine nutrient availability in a Mediterranean forest stream. *Aquatic Sciences*, **73**, 485–497.

Schindler, D.E., Carpenter, S.R., Cole, J.J., Kitchell, J.F. & Pace, M.L. (1997) Influence of food web structure on carbon exchange between lakes and the atmosphere. *Science*, **277**, 248–251.

Schmitz, O.J., Hawlena, D. & Trussell, G.C. (2010) Predator control of ecosystem nutrient dynamics. *Ecology Letters*, **13**, 1199–1209.

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

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

Seddon, P.J. (2010) From reintroduction to assisted colonization: moving along the conservation translocation spectrum. *Restoration Ecology*, **18**, 796–802.

Sestrich, C.M., McMahon, T.E. & Young, M.K. (2011) Influence of fire on native and nonnative salmonid populations and habitat in a western montana basin. *Transactions of the American Fisheries Society*, **140**, 136–146.

Short, T.M. & Holomuzki, J.R. (1992) Indirect effects of fish on foraging behaviour and leaf processing by the isopod *Lirceus fontinalis*. *Freshwater Biology*, **27**, 91–97.

Short, R.A. & Smith, S.L. (1989) Seasonal comparison of leaf processing in a Texas stream. *American Midland Naturalist*, **121**, 219–224.

Shulman, R.S. & Chase, J.M. (2007) Increasing isolation reduces predator:prey species richness ratios in aquatic food webs. *Oikos*, **116**, 1581–1587.

Shurin, J.B., Borer, E.T., Seabloom, E.W., Anderson, K., Blanchette, C.A., Broitman, B., Cooper, S.D. & Halpern, B.S. (2002) A cross-ecosystem comparison of the strength of trophic cascades. *Ecology Letters*, **5**, 785–791.

Sih, A., Bolnick, D.I., Luttbeg, B., Orrock, J.L., Peacor, S.D., Pintor, L.M., Preisser, E., Rehage, J.S. & Vonesh, J.R. (2010) Predator-prey naïveté, antipredator behavior, and the ecology of predator invasions. *Oikos*, **119**, 610–621.

Smock, L.A. (1980) Relationships between body size and biomass of aquatic insects. *Freshwater Biology*, **10**, 375–383.

Snyder, W.E. & Ives, A.R. (2001) Generalist predators disrupt biological control by a specialist parasitoid. *Ecology*, **82**, 705–716.

de Sostoa, A., Caiola, N., Vinyoles, D. & Casals, F. (2006) *Diagnosi de les poblacions de peixos del Parc Natural de Sant Llorenç del Munt i l'Obac.* Diputació de Barcelona, Oficina Tècnica de Parcs Naturals, Barcelona.

Soulé, M.E., Estes, J.A., Berger, J. & Martinez del Rio, C. (2003) Ecological effectiveness: conservation goals for interactive species. *Conservation Biology*, **17**, 1238–1250.

Spencer, C.N., Gabel, K.O. & Hauer, F.R. (2003) Wildfire effects on stream food webs and nutrient dynamics in Glacier National Park, USA. *Forest Ecology and Management*, **178**, 141–153.

Staddon, P., Lindo, Z., Crittenden, P.D., Gilbert, F. & Gonzalez, A. (2010) Connectivity, non-random extinction and ecosystem function in experimental metacommunities. *Ecology Letters*, **13**, 543–552.

Stanley, E., Buschman, D., Boulton, A.J., Grimm, N.B. & Fisher, S.G. (1994) Invertebrate resistance and resilience to intermittency in a desert stream. *American Midland Naturalist*, **131**, 288–300.

Surasinghe, T. & Baldwin, R.F. (2014) Ghost of land-use past in the context of current land cover: evidence from salamander communities in streams of Blue Ridge and Piedmont ecoregions. *Canadian Journal of Zoology*, **92**, 527–536.

Sutherland, W.J., Freckleton, R.P., Godfray, H.C.J., Beissinger, S.R., Benton, T., Cameron, D.D., Carmel, Y., Coomes, D.A., Coulson, T., Emmerson, M.C., Hails, R.S., Hays, G.C., Hodgson, D.J., Hutchings, M.J., Johnson, D., Jones, J.P.G., Keeling, M.J., Kokko, H., Kunin, W.E., Lambin, X., Lewis, O.T., Malhi, Y., Mieszkowska, N., Milner-Gulland, E.J., Norris, K., Phillimore, A.B., Purves, D.W., Reid, J.M., Reuman, D.C., Thompson, K., Travis, J.M.J., Turnbull, L.A., Wardle, D.A. & Wiegand, T. (2013) Identification of 100 fundamental ecological questions. *Journal of Ecology*, **101**, 58–67.

Tachet, H., Richoux, P., Bournard, M. & Usseglio-Polatera, P. (2010) *Invertébrés d'eau douce: systématique, biologie, écologie.* CNRS éditions, Paris.

Tank, J.L., Rosi-Marshall, E.J., Griffiths, N.A., Entrekin, S.A. & Stephen, M.L. (2010) A review of allochthonous organic matter dynamics and metabolism in streams. *Journal of the North American Benthological Society*, **29**, 118–146.

Tant, C.J., Rosemond, A.D. & First, M.R. (2013) Stream nutrient enrichment has a greater effect on coarse than on fine benthic organic matter. *Freshwater Science*, **32**, 1111–1121.

Terborgh, J. & Estes, J.A. (2010) *Trophic cascades: predators, prey, and the changing dynamics of nature*. Island Press, Washington, DC.

Thompson, R.M., Brose, U., Dunne, J.A., Hall, R.O., Hladyz, S., Kitching, R.L., Martinez, N.D., Rantala, H., Romanuk, T.N., Stouffer, D.B. & Tylianakis, J.M. (2012) Food webs: reconciling the structure and function of biodiversity. *TRENDS in Ecology and Evolution*, 1–9.

Thompson, R.M. & Townsend, C.R. (2000) Is resolution the solution?: the effect of taxonomic resolution on the calculated properties of three stream food webs. *Freshwater Biology*, **44**, 413–422.

Tiegs, S.D., Peter, F.D., Robinson, C.T., Uehlinger, U. & Gessner, M.O. (2008) Leaf decomposition and invertebrate colonization responses to manipulated litter quantity in streams. *Journal of the North American Benthological Society*, **27**, 321–331.

Timoner, X., Acuña, V., Von Schiller, D. & Sabater, S. (2012) Functional responses of stream biofilms to flow cessation, desiccation and rewetting. *Freshwater Biology*, **57**, 1565–1578.

Townsend, C.R. (1989) The patch dynamics concept of stream community ecology. *Journal of the North American Benthological Society*, **8**, 36–50.

Treplin, M. & Zimmer, M. (2012) Drowned or dry: a cross-habitat comparison of detrital breakdown processes. *Ecosystems*, **15**, 477–491.

Vance-Chalcraft, H.D., Rosenheim, J.A., Vonesh, J.R., Osenberg, C.W. & Sih, A. (2007) The influence of intraguild predation on prey suppression and prey release: a meta-analysis. *Ecology*, **88**, 2689–2696.

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

Vanni, M. (2010) When and where do fish have strong effects on stream ecosystem processes? *American Fisheries Society Symposium*, **73**, 1–8.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R. & Cushing, C.E. (1980) The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, **37**, 130–137.

Vaz, P.G., Merten, E.C., Warren, D.R., Durscher, K., Tapp, M., Robinson, C.T., Rego, F.C. & Pinto, P. (2015) Fire meets inland water via burned wood: and then what? *Freshwater Science*, **9**, 1468–1481.

Vaz, P.G., Merten, E.C., Warren, D.R., Robinson, C.T., Pinto, P. & Rego, F.C. (2013) Which stream wood becomes functional following wildfires? *Ecological Engineering*, **54**, 82–89.

Verkaik, I. (2010) *Wildfire effects on macroinvertebrate communities in Mediterranean streams*. PhD tesis, Universidad de Barcelona, Barcelona.

Verkaik, I., Rieradevall, M., Cooper, S.D., Melack, J.M., Dudley, T.L. & Prat, N. (2013a) Fire as a disturbance in mediterranean climate streams. *Hydrobiologia*, **719**, 353–382.

Verkaik, I., Vila-Escalé, M., Rieradevall, M. & Prat, N. (2013b) Seasonal drought plays a stronger role than wildfire in shaping macroinvertebrate communities of Mediterranean streams. *International Review of Hydrobiology*, **98**, 1–13.

Vieira, N.K.M., Barnes, T.R. & Mitchell, K.A. (2011) Effects of wildfire and postfire floods on stonefly detritivores of the Pajarito Plateau, New Mexico. *Western North American Naturalist*, **71**, 257–270.

Vieira, N.K.M., Clements, W.H., Guevara, L.S. & Jacobs, B.F. (2004) Resistance and resilience of stream insect communities to repeated hydrologic disturbances after a wildfire. *Freshwater Biology*, **49**, 1243–1259.

Vieira, N.K.M., Poff, N.L., Carlisle, D.M., Moulton II, S.R., Koski, M.L. & Kondratieff, B.C. (2006) *A database of lotic invertebrate traits for North America.* U.S. Geological Survey Data Series 187, http://pubs. water.usgs.gov/ds187.

Vila-Escalé, M. (2009) *Efectes d'un incendi forestal en una riera mediterrànea (Sant Llorenç del Munt, 2003)*. PhD thesis, Universidad de Barcelona, Barcelona.

Vila-Escalé, M., Vegas-Vilarrúbia, T. & Prat, N. (2007a) Release of polycyclic aromatic compounds into a Mediterranean creek (Catalonia, NE Spain) after a forest fire. *Water Research*, **41**, 2171–2179.

Vila-Escalé, M., Vegas-Vilarrúbia, T., Verkaik, I., Rieradevall, M. & Prat, N. (2007b) Changes in the aquatic habitat in Gallifa gully after a forest fire. *VI Trobada d'Estudiosos de Sant Llorenç del Munt i l'Obac* (eds J. Hernández, J. Melero, & J. Grau), pp. 199–203. Diputació de Barcelona, Barcelona.

Vila-Escalé, M., Verkaik, I., Vegas-Vilarrúbia, T., Rieradevall, M. & Prat, N. (2007c) Evolution of the community of macroinvertebrates in a Mediterranean river after a forest fire. *VI Trobada d'Estudiosos de Sant Llorenç del Munt i l'Obac* (eds J. Hernández, J. Melero, & J. Grau), pp. 205–209. Diputació de Barcelona, Barcelona.

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

Vitule, J.R.S., Freire, C.A., Vazquez, D.P., Nuñez, M.A. & Simberloff, D. (2012) Revisiting the potential conservation value of non-native species. *Conservation Biology*, **26**, 1153–1155.

Wallace, J.B., Eggert, S.L., Meyer, J.L. & Webster, J.R. (1997) Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science*, **277**, 102–104.

Warren, P.H. & Gaston, K.J. (1992) Predator-prey ratios: a special case of a general pattern? *Philosophical Transactions of the Royal Society B: Biological Sciences*, **338**, 113–130.

Webster, J.R. & Benfield, E.F. (1986) Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology and Systematics*, **17**, 567–594.

Werner, E.E. & Hall, D.J. (1974) Optimal foraging and the size selection of prey by the bluegill sunfish (*Lepomis macrochirus*). *Ecology*, **55**, 1042–1052.

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

Whitney, J.E., Gido, K.B., Pilger, T.J., Propst, D.L. & Turner, T.F. (2015) Consecutive wildfires affect stream biota in cold- and warmwater dryland river networks. *Freshwater Science*, **34**, 1510–1526.

Wiens, J.A. (2002) Riverine landscapes: taking landscape ecology into the water. *Freshwater Biology*, **47**, 501–515.

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

Williams, D.D. (2006) *The biology of temporary waters*. Oxford University Press, New York.

Williams, D.D. & Coad, B.W. (1979) The ecology of temporary streams III. Temporary stream fishes in Southern Ontario, Canada. *Internationale Revue der gesamten Hydrobiologie und Hydrographie*, **64**, 285–308.

Williams, D.D. & Hynes, H.B.N. (1976) The ecology of temporary streams. I. The fauna of two canadian streams. *Internationale Revue der gesamten Hydrobiologie*, **61**, 761–787.

Williams, D.D. & Hynes, H.B.N. (1977) The ecology of temporary streams II. General remarks on temporary streams. *Internationale Revue der gesamten Hydrobiologie und Hydrographie*, **62**, 53–61.

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

Wilson, J.B. (1996) The myth of constant predator:prey ratios. *Oecologia*, **106**, 272–276.

Winkelmann, C., Worischka, S., Koop, J.H.E. & Benndorf, J. (2007) Predation effects of benthivorous fish on grazing and shredding macroinvertebrates in a detritus-based stream food web. *Limnologica - Ecology and Management of Inland Waters*, **37**, 121–128.

Wondzell, S.M. & King, J.G. (2003) Postfire erosional processes in the Pacific Northwest and Rocky Mountain regions. *Forest Ecology and Management*, **178**, 75–87.

Woodward, G., Blanchard, J., Lauridsen, R.B., Edwards, F.K., Jones, J.I., Figueroa, D., Warren, P.H. & Petchey, O.L. (2010) Individual-based food webs: species identity, body size and sampling effects. *Advances in Ecological Research*, **43**, 211–266.

Woodward, G., Gessner, M.O., Giller, P.S., Gulis, V., Hladyz, S., Lecerf, A., Malmqvist, B., McKie, B.G., Tiegs, S.D., Cariss, H., Dobson, M., Elosegi, A., Ferreira, V., Graça, M.A.S., Fleituch, T., Lacoursière, J.O., Nistorescu, M., Pozo, J., Risnoveanu, G., Schindler, M., Vadineanu, A., Vought, L.B.-M. & Chauvet, E. (2012) Continental-scale effects of nutrient pollution on stream ecosystem functioning. *Science*, **336**, 1438–1440.

Woodward, G., Papantoniou, G., Edwards, F.K. & Lauridsen, R.B. (2008) Trophic trickles and cascades in a complex food web: impacts of a keystone predator on stream community structure and ecosystem processes. *Oikos*, **117**, 683–692.

Woodward, G. & Warren, P.H. (2007) Body size and predatory interactions in freshwaters: scaling from individuals to communities. *Body Size: the structure and function of aquatic ecosystems* (eds Alan G. Hildrew, D.G. Raffaelli, & R. Edmonds-Brown), pp. 98–117. Cambridge Universiy Press, Cambridge.

Wootton, J.T. (2002) Indirect effects in complex ecosystems: recent progress and future challenges. *Journal of Sea Research*, **48**, 157–172.

Wrona, F.J. & Dixon, R.W.J. (1991) Group size and predation risk: a field analysis of encounter and dilution effects. *The American Naturalist*, **137**, 186–201.

Young, R.G., Matthaei, C.D. & Townsend, C.R. (2008) Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. *Journal of the North American Benthological Society*, **27**, 605–625.

Zar, J.H. (2010) *Biostatistical Analysis*, 5th ed. Prentice Hall, New Jersey.

Supporting information

This section comprises supporting information for Chapter 1, Chapter 2, and Chapter 3.

Appendix A provides the original data regarding macroinvetebrate community composition and abundance, periphyton primary production, and *Barbus meridionalis* gut contents from the mesocosm experiment performed for Chapter 2 and Chapter 3.

Appendix B provides the original publications of Chapter 1 and Chapter 2.

Appendix A

This appendix provides the original data regarding Chapter 2 and Chapter 3. It comprises 2 tables.

Table S.1 Taxa abundance and periphyton primary production data for the three experimental treatments. Taxa are sorted by decreasing abundance in the treatment without barbels. Category: 1 = primary consumer; 2 = secondary consumer.

	Category	Without top predator		Low top predator density		High top predator density	
Taxa (ind m^{-2})		mean	SE	mean	SE	mean	SE
Tanytarsus sp.	1	4882.5	890.2	3334.3	817.0	1955.8	543.8
Zavrelimyia sp.	2	2433.7	330.7	1928.2	153.3	1132.3	135.9
Cricotopus sp.	1	2003.3	475.4	1468.0	243.2	831.2	132.2
Procladius sp.	2	1203.6	352.1	283.9	108.5	361.3	172.1
Habrophlebia sp.	1	1114.3	209.6	708.5	57.6	456.2	33.6
Cladocera	1	1034.7	247.5	557.8	97.2	256.3	44.4
Dicrotendipes sp.	1	888.1	214.6	1644.4	372.5	948.9	162.0
Corynoneura sp.	1	673.6	148.6	932.4	123.9	634.2	100.1
Gyraulus sp.	1	651.8	140.4	667.2	100.9	519.5	114.4
Paratanytarsus sp.	1	560.7	255.9	878.1	198.2	506.5	186.4
Microtendipes sp.	1	546.3	88.0	714.4	284.9	1329.5	988.3
Radix sp.	1	449.6	92.6	257.1	74.0	136.3	31.4
Ostracoda	1	396.7	67.9	146.1	34.7	159.0	40.5
Hydra sp.	2	373.2	87.9	375.1	105.6	105.1	28.3
Nanocladius sp.	1	321.9	63.5	437.0	119.1	232.6	38.5
Helobdella stagnalis	2	279.5	47.9	175.8	48.1	201.9	43.5
Oligochaeta	1	275.9	93.8	238.3	47.1	81.9	17.7
Phaenopsectra sp.	1	221.4	106.8	216.9	87.3	98.8	39.5
Chironomus sp.	1	188.9	95.5	131.2	65.2	36.3	27.6
Chalcolestes viridis	2	175.5	24.2	59.0	5.1	39.6	4.4
Baetis sp.	1	148.6	54.1	146.5	15.1	34.6	16.2
Stictonectes sp.	2	132.9	26.2	46.5	11.4	32.6	4.4
Cloeon sp.	1	116.5	29.0	17.4	5.7	30.5	7.6
Acentrella sp.	1	109.5	38.2	126.5	26.0	64.5	21.8
Caenis sp.	1	94.9	21.7	75.5	9.7	64.8	15.2
Parasigara sp.	2	81.3	16.9	25.8	5.8	10.7	3.3
Rheotanytarsus sp.	1	71.9	71.9	371.9	306.9	43.7	25.3
Cladotanytarsus sp.	1	70.8	70.8	0.0	0.0	0.0	0.0

	Category	Without top predator		Low top predator density		High top predator density	
Taxa (ind m^{-2})		mean	SE	mean	SE	mean	SE
Polypedilum sp.	1	62.4	26.4	48.3	42.7	126.5	77.4
Chaoborus sp.	2	61.1	24.4	4.2	1.8	0.7	0.7
Copepoda	1	40.1	16.4	77.3	15.5	20.1	12.2
Sympetrum sp.	2	37.5	6.9	33.3	7.5	13.9	3.3
Dasyheleinae	1	35.9	12.3	9.7	3.0	8.9	3.7
Pelophylax perezi	1	23.6	11.3	4.2	1.5	10.4	4.9
Hidracarina	2	20.8	11.7	27.0	24.8	13.9	9.4
Haliplus sp.	1	17.4	16.6	0.0	0.0	4.2	2.1
Cyrnus sp.	2	16.8	8.8	10.5	4.4	7.6	2.7
Agabus sp.	2	15.3	6.7	2.1	1.0	1.4	0.9
Terrestrial inv.	-	13.2	12.4	2.8	1.5	2.1	1.5
Dugesia sp.	2	12.4	11.0	2.1	1.5	6.9	5.6
Physella sp.	1	9.7	6.4	7.6	2.3	2.8	1.5
Aeshna sp.	2	9.0	3.9	4.9	2.3	1.4	0.9
Dixa sp.	1	8.7	5.4	2.8	1.6	5.5	2.6
Coenagrionidae	2	7.6	5.5	0.7	0.7	1.4	0.9
Notonecta sp.	2	7.6	5.4	4.1	1.8	1.4	0.9
Yola bicarinata	1	6.8	5.5	10.5	2.8	6.2	2.8
Oulimnius sp.	2	4.9	2.9	14.5	3.6	3.4	1.8
Ancylus fluviatilis	1	3.6	1.8	0.7	0.7	4.1	2.2
Deronectes sp.	2	2.8	2.1	0.0	0.0	0.0	0.0
Elmis sp.	1	2.8	2.8	0.0	0.0	0.0	0.0
Brachytron pratense	2	2.1	1.0	0.0	0.0	0.0	0.0
Gyrinus sp.	2	2.1	1.0	3.5	1.1	0.7	0.7
Nebrioporus sp.	2	2.1	1.5	2.8	1.5	2.1	1.1
Pisidium sp.	1	1.9	1.4	5.6	3.8	17.4	11.7
Psectrocladius sp.	1	1.4	1.0	18.1	18.1	42.4	28.8
Anax imperator	2	1.4	0.9	1.4	0.9	0.7	0.7
Simuliidae	1	1.4	1.4	1.4	0.9	4.4	2.8
Hemerodromiinae	2	0.7	0.7	3.5	2.1	0.0	0.0
Hydrometra sp.	2	0.7	0.7	2.3	2.3	0.0	0.0
Microvelia sp.	2	0.7	0.7	0.0	0.0	0.7	0.7
Esolus sp.	1	0.7	0.7	0.0	0.0	1.4	1.4
Ilybius sp.	2	0.7	0.7	0.0	0.0	0.0	0.0
Limnephilus sp.	1	0.7	0.7	0.0	0.0	0.7	0.7
Pyralydae	1	0.7	0.7	0.7	0.7	0.0	0.0
Serratella sp.	1	0.7	0.7	0.0	0.0	0.7	0.7
Sialis sp.	2	0.7	0.7	0.0	0.0	0.0	0.0
Tinodes sp.	1	0.7	0.7	0.7	0.7	3.5	2.8

	Category	Without top predator		Low top predator density		High top predator density	
Taxa (ind m^{-2})		mean	SE	mean	SE	mean	SE
Stratiomyidae	1	0.7	0.7	0.0	0.0	0.7	0.7
Atherix sp.	2	0.0	0.0	0.7	0.7	0.0	0.0
Brillia sp.	1	0.0	0.0	0.0	0.0	8.5	8.5
Limnophyes sp.	1	0.0	0.0	40.0	27.3	24.1	24.1
Mesovelia vittigera	2	0.0	0.0	0.6	0.6	0.0	0.0
Micropsectra sp.	1	0.0	0.0	0.0	0.0	30.6	30.6
Muscidae	2	0.0	0.0	0.0	0.0	0.7	0.7
Normandia sp.	1	0.0	0.0	0.7	0.7	0.0	0.0
Parametriocnemus sp.	1	0.0	0.0	0.0	0.0	17.1	17.1
Rheocricotopus sp.	1	0.0	0.0	12.0	12.0	17.4	17.4
Stictotarsus sp.	2	0.0	0.0	0.0	0.0	0.7	0.7

Primary production		mean	SE	mean	SE	mean	SE
Chl-*a* mg m^{-2} d^{-1}	-	332.9	37.5	601.5	53.9	528.9	35.8

Table S.2 *Barbus meridionalis* gut contents: abundance (%) and frequency of occurrence (%) of macroinvertebrate taxa. The different prey items are ordered by frequency of occurrence.

Taxa	Abundance (%)	Frequency of occurrence (%)
Cricotopus spp.	51.1	100.0
Habrophlebia sp.	15.1	100.0
Zavrelimyia sp.	10.9	94.4
Stictonectes sp.	3.1	88.9
Corynoneura sp.	3.6	83.3
Microtendipes sp.	2.8	83.3
Parasigara sp.	3.5	61.1
Agabus sp.	1.0	61.1
Procladius sp.	0.9	44.4
Dicrotendipes sp.	1.2	38.9
Tanytarsus sp.	0.3	33.3
Chalcolestes viridis	0.3	33.3
Gyraulus sp.	1.7	27.8
Cladocera	0.4	27.8
Nebrioporus sp.	0.4	22.2
Baetis sp.	0.3	22.2
Gyrinus sp.	0.2	22.2
Tinodes sp.	0.3	16.7
Cloeon sp.	0.2	16.7
Sympetrum sp.	0.2	16.7
Terrestrial inv.	0.2	11.1
Aeshna sp.	0.2	11.1
Nanocladius sp.	0.1	11.1
Paratanytarsus sp.	0.1	11.1
Coenagrionidae	< 0.1	11.1
Yola bicarinata	< 0.1	11.1
Physella sp.	0.7	5.6
Radix sp.	0.2	5.6
Ostracoda	0.2	5.6
Stictochironomus sp.	0.1	5.6
Haliplus sp.	0.1	5.6
Polypedilum sp.	< 0.1	5.6
Phaenopsectra sp.	< 0.1	5.6
Brillia sp.	< 0.1	5.6
Helobdella stagnalis	< 0.1	5.6
Rheotanytarsus sp.	< 0.1	5.6
Parametriocnemus sp.	< 0.1	5.6
Oulimnius sp.	< 0.1	5.6

Appendix B

This Appendix provides the original publications of Chapter 1 and Chapter 2.

Rodríguez-Lozano, P., Rieradevall, M., Rau, M.A. & Prat, N. (2015) Long-term consequences of a wildfire for leaf-litter breakdown in a Mediterranean stream. Freshwater Science, 34(4): 1482–1493. doi:10.1086/683432

Rodríguez-Lozano, P., Verkaik, I., Rieradevall, M. & Prat, N. (2015) Small but powerful: top predator local extinction affects ecosystem structure and function in an intermittent stream. *PLoS ONE,* 10(2): e0117630. doi:10.1371/journal.pone.0117630

Long-term consequences of a wildfire for leaf-litter breakdown in a Mediterranean stream

Pablo Rodríguez-Lozano[1,3], **Maria Rieradevall**[1,4], **Marius Andrei Rau**[2,5], **and Narcís Prat**[1,6]

[1]Freshwater Ecology and Management (F.E.M.) Research Group, Departament d'Ecologia, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain
[2]'Alexandru Ioan Cuza' University of Iasi, Faculty of Biology, Laboratory of Hydrobiology, Iasi, Romania

Abstract: Wildfire frequency and intensity are expected to increase as a result of climate change, but few studies have assessed the effects of wildfires on stream ecosystem processes. The aim of our study was to examine the long-term responses of leaf-litter breakdown to wildfire in a Mediterranean stream. Riparian canopy cover was reduced by a fire, resulting in higher temperatures and light levels and reduced leaf-litter inputs in the stream 8 y after the fire. Benthic invertebrate communities recovered quickly after the fire, and the abundances of different functional feeding groups (FFGs), including shredders, were not different between streams affected and unaffected by the fire after >2 postfire years. We compared microbially mediated (fine-mesh bags) and total breakdown rates (coarse-mesh bags) of *Populus alba* leaf litter incubated in the stream affected by the wildfire and a neighboring control stream. Microbial and total leaf-litter breakdown were faster in the stream affected by fire. Faster microbially induced litter decomposition in the fire-affected stream could be attributed almost entirely to increased water temperatures, but total litter breakdown rates were enhanced by higher shredder abundance in coarse-mesh leaf bags in the fire-affected than control stream. Lower leaf-litter inputs in the fire-affected than in the control stream probably led to lower benthic organic matter levels, leading to increased shredder aggregation in leaf packs, thereby accelerating leaf breakdown rates. Our study indicates that past wildfires can modify key ecosystem processes, such as leaf decomposition, in the long term in Mediterranean streams.

Key words: wildfire, leaf decomposition, Mediterranean streams, long-term effects, litterfall, shredders, ecosystem process, landscape disturbance, ecosystem function, resource tracking, climate change

Wildfires are expected to increase in frequency and intensity because of climate change and changes in land use and management (Bowman et al. 2011, Dury et al. 2011, IPCC 2014). Fire is recognized as one of the main factors that shape ecosystems (Bond and Keeley 2005, Cowling et al. 2005), but few investigators have studied its effects on stream ecosystem processes (but see Robinson et al. 2005, Betts and Jones 2009, Koetsier et al. 2010). Leaf-litter inputs and decomposition fuel many stream food webs, and the authors of a recent review recommended conducting additional studies on fire effects on the key ecosystem process of leaf-litter breakdown (Verkaik et al. 2013a). Experimental studies suggest that decomposition rates of burned leaves do not differ from (Gama et al. 2007), or are faster than (Vieira et al. 2011), those of unburned leaves. Koetsier et al. (2010), authors of the only study we know of fire effects on stream leaf-litter breakdown in the long term, found that litter decomposition rates were faster in fire-affected than in unaffected reference streams in a temperate forest. These results provide evidence of the need for additional studies on fire effects on stream leaf-litter breakdown in other biomes, such as Mediterranean regions, where fire is common.

Wildfires could stimulate leaf decomposition mediated by microbes by reducing riparian canopy cover, thereby increasing water temperatures (Koetsier et al. 2010, Boyero et al. 2011, Martínez et al. 2014, Cooper et al. 2015). Reductions in canopy cover also increase light availability, potentially enhancing microbial decomposition via increases in algal production on leaf litter (Danger et al. 2013). Moreover, algal development on leaves could increase litter palatability, accelerating litter breakdown me-

E-mail addresses: [3]pablorodriguezlozano@gmail.com; [4]mrieradevall@ub.edu; [5]marius.rau88@gmail.com; [6]nprat@ub.edu

---

DOI: 10.1086/683432. Received 30 January 2015; Accepted 11 June 2015; Published online 27 August 2015.
Freshwater Science. 2015. 34(4):1482–1493. © 2015 by The Society for Freshwater Science.

This content downloaded from 23.235.32.0 on Fri, 13 Nov 2015 14:49:38 PM
All use subject to JSTOR Terms and Conditions

diated by macroinvertebrate activity (Lagrue et al. 2011). In contrast, fire-induced reductions in canopy cover could increase ultraviolet radiation levels, thereby reducing leaf-litter mass loss (Pancotto et al. 2003, Dieter et al. 2011). Wildfires also could modify microbially mediated litter decomposition rates by increasing dissolved nutrient concentrations, which would accelerate decomposition (Woodward et al. 2012), and by increasing the scour and deposition of fine sediment, which could reduce (Benfield et al. 2001, Lecerf and Richardson 2010) or increase (Piggott et al. 2012) decomposition. However, these effects of fire on nutrients and sediment are often short-lived, so litter decomposition may not be affected over the long term by these agents (Benfield et al. 2001, Earl and Blinn 2003, Vila-Escalé 2009, Lecerf and Richardson 2010, Verkaik et al. 2013a).

Macroinvertebrate-mediated leaf breakdown could decrease postfire if shredding invertebrate density decreases (Molles 1982, Vieira et al. 2004, Oliver et al. 2012, Verkaik et al. 2013a, Cooper et al. 2015). Fire-mediated decreases in shredder density can be attributed to decreased leaf-litter inputs and retention postfire and to the vulnerability of shredders to scouring floods induced by fire (Verkaik et al. 2013a, Cooper et al. 2015). Observational (Boulton and Lake 1992, Friberg 1997, González and Graça 2005) and experimental (Richardson 1991, Dobson and Hildrew 1992, Wallace et al. 1997) studies indicate that shredder densities in streams are related to organic matter availability. Knowledge about the effects of wildfires on organic matter inputs to streams is limited, but terrestrial subsidies of leaf litter to streams appear to increase immediately postfire and decrease over the following years (Britton 1990, Jackson et al. 2012). On the other hand, increased stream temperatures postfire could increase shredder activity. Wildfires may have little effect on leaf breakdown mediated by macroinvertebrate activity in the long term because the abundance and taxonomic and functional composition of macroinvertebrate communities in Mediterranean streams often recover quickly after wildfires (Vila-Escalé 2009, Verkaik et al. 2013a, b).

The aim of our study was to assess the long-term (8 y) effects of a wildfire on leaf-litter breakdown in a Mediterranean stream. We differentiated between fire effects on microbial and overall leaf decomposition to better understand the mechanisms accounting for wildfire effects on this ecosystem process. We conducted a decomposition experiment using *Populus alba* leaves enclosed in coarse- or fine-mesh bags incubated in a stream affected by fire and in a neighboring control stream. To assess some of the key factors potentially mediating the effects of the wildfire on stream litter breakdown, we monitored the riparian canopy cover postfire, temperature and light levels during the decomposition experiment, litterfall input over 1 y encompassing the decomposition experiment, and the abundances of macroinvertebrate functional feeding groups (FFGs) in streams draining burned and unburned catchments over the first 5 y postfire and in leaf bags during the experiment (8 y postfire). We hypothesized that 8 y after a wildfire: 1) microbial leaf decomposition would be faster in the fire-affected than control stream because of increases in water temperature and 2) macroinvertebrate-mediated leaf breakdown would be similar between the fire-affected and control streams because of the rapid recovery of macroinvertebrate communities, including shredder densities, postfire.

METHODS

Study area

We worked in 2 adjacent 1st-order streams, separated by <5 km. The fire-affected stream was Vall d'Horta stream (lat 41°39′15″N, long 2°4′13″E), and the control stream was Castelló stream (lat 41°40′42″N, long 2°1′49″E). The streams are in the protected area of Sant Llorenç del Munt i l'Obac Natural Park (50 km inland from Barcelona, northeastern Spain). This area is characterized by a calcareous geology and a Mediterranean climate, with mild winters and warm springs and summers. Rainfall is irregular and intense, occurs primarily in winter but also in spring and autumn, and summers are normally very dry. The protected area is dominated by Holm Oak (*Quercus ilex* L.) and Aleppo pine (*Pinus halepensis* Miller) forests and Mediterranean shrubs (for a detailed description see Bonada et al. 2007b, Verkaik et al. 2013b). In August 2003, a wildfire burned 4543 ha of forest in the Natural Park and surrounding areas.

Both streams are tributaries of the Ripoll River in the Besòs basin, and their catchments are mainly forested, dominated by Aleppo pine with small areas of cereal cultivation (<15%). The selected 100-m reach in each stream was <2 m wide and <50 cm deep, with a bedrock stream bed. In the 2003 fire, 62.1% of the catchment of the fire-affected stream, including its riparian zone, was burned. Riparian and upland forests have not recovered completely at this site. The control stream was unaffected by the wildfire and continues to be shaded by a well-developed canopy (Table 1). In summer 2012, riparian vegetation at the fire-affected site consisting of 25 to 50% trees, 25% shrubs, and 25 to 50% annual herbs, whereas the control reach was lined by a well developed riparian forest (>75% trees) with understory shrubs. These 2 reaches, 1 additional control reach, and 5 additional fire-affected reaches were monitored by Verkaik et al. (2013b) for 5 y postfire, and we reanalyzed data from that study to estimate the responses of invertebrate FFGs to fire.

Canopy cover and physicochemical measurements

Riparian canopy cover was estimated annually as part of a riparian forest quality index (QBR index; Munné et al. 2003) at the control and fire-affected sites from 2 mo

This content downloaded from 23.235.32.0 on Fri, 13 Nov 2015 14:49:38 PM
All use subject to JSTOR Terms and Conditions

Table 1. Mean (±1 SE) values of characteristics of the control and fire-affected sites during the litter decomposition experiment (23 November 2011–15 March 2012), and results of analyses of variance comparing characteristics between control and fire-affected sites. PAR = photosynthetically active radiation.

	Control	Fire affected	*F*	*p*
Elevation (m asl)	451	485	–	–
Catchment area (ha)	290	510	–	–
Burned area (%)	0	62.1	–	–
Discharge (L/s)	5.5 ± 1.5	18.6 ± 7.7	2.8	0.12
Water temperature (°C)	5.6 ± 0.2	8.2 ± 0.2	77	<0.001
PAR (μmol m^{-2} s^{-1})	10.1 ± 0.5	21.5 ± 1.0	264	<0.001
pH	8.16 ± 0.02	8.09 ± 0.02	4.0	0.07
Conductivity (μS/cm)	492 ± 2	485 ± 2	4.7	0.06
DO (% saturation)	86.8 ± 0.3	89.3 ± 0.2	4.8	0.09
NH_4^+-N (μg/L)	23 ± 1	24 ± 1	1.7	0.22
NO_3^--N (μg/L)	124 ± 14	640 ± 13	744	<0.001
PO_4^{3-}-P (μg/L)	<10	<10	–	–

after the fire until summer 2012. We reanalyzed the canopy cover data collected by Verkaik et al. (2013b) for 2 control and 6 fire-affected reaches over the first 5 y postfire. We also made physicochemical measurements throughout the litter-decomposition experiment (*n* = 6 times). We measured dissolved O_2, pH, and conductivity in situ with a Multiline P4 WTW meter (YSI; Yellow Springs, Ohio), and we estimated discharge from mean depth, transect width, and water velocity with a flow meter (miniAir®; Schiltknecht, Gossau, Switzerland). We collected water samples by hand, filtered them through GF/F Whatman filters, and analyzed them for soluble nutrients. We measured NH_4^+-N and soluble reactive P (PO_4^{3-}-P) with spectrophotometric methods (Murphy and Riley 1962) and NO_3^--N with ionic chromatography methods (EPA method 9056, USEPA 1993; UV/V KONTRON model 332; Kontron AG, Zürich, Switzerland). We deployed submersible temperature/light data loggers (HOBO Pendant; Onset Computer Corporation, Bourne, Massachusetts) under water to record water temperature and light intensity every 10 min at each site throughout the leaf-bag experiment.

Benthic macroinvertebrate communities

We compared the abundances of benthic macroinvertebrate FFGs at 2 control and 6 fire-affected sites over the first 5 y postfire by reanalyzing the data collected by Verkaik et al. (2013b). At each site and time, macroinvertebrate kick samples were collected with a 250-μm net from all extant habitats over a standard 4-min period. Macroinvertebrate samples were processed as outlined below for macroinvertebrates collected from leaf bags.

Litterfall input

We measured vertical litterfall inputs with 0.28-m^2 traps (3 at the fire-affected site and 3 at the control site) placed in the riparian zone (Pozo et al. 1997). Traps were made of rigid baskets 60 cm in depth and lined with a 1-mm mesh, allowing us to retain all coarse particulate organic matter (CPOM) while allowing water to escape during rain events. Litter in traps was removed 12 times during a year (4 October 2011–4 October 2012), with increased sampling frequency in autumn. We oven-dried litter (60°C, 72 h), and sorted it as leaves, bark and twigs, or fruits and flowers. We weighed and combusted (500°C, 12 h) this material to measure ash-free dry mass (AFDM).

Leaf-litter decomposition experiment

We conducted the leaf-bag experiment in autumn–winter (23 November 2011–15 March 2012). We collected leaves of white poplar (*Populus alba* L.) in October 2011 just after abscission, and air-dried them at room temperature to constant mass. Leaf bags (15 × 20 cm) containing 3 g of leaves (SE = 0.06 g) were made of 2 mesh sizes: coarse (10 mm) and fine (250 μm). Coarse-mesh bags allowed macroinvertebrate colonization, thus, more closely simulated natural leaf-litter breakdown processes, whereas fine-mesh bags excluded virtually all macroinvertebrates, thereby allowing us to assess the relative contribution of microbial (fungi and bacteria) activity to litter decomposition (see Young et al. 2008).

We deployed 30 bags of each mesh type at the fire-affected and control sites in pools because leaves accumulated in the pools of these streams. We placed 12 additional bags in the control site for 24 h to correct for

This content downloaded from 23.235.32.0 on Fri, 13 Nov 2015 14:49:38 PM
All use subject to JSTOR Terms and Conditions

initial leaf mass losses resulting from leaching and accidental transport losses (Gessner et al. 1999). We removed 6 litter bags of each type (coarse and fine mesh) from each site 5, 12, 26, 58, and 113 d after deployment. At retrieval, we placed litter bags individually in Ziploc® bags and transported them in refrigerated containers to the laboratory, where we processed them immediately. We washed the material in each litter bag, collected invertebrates on a 250-µm sieve, and preserved them in 70% ethanol. We oven-dried (60°C, 72 h) and combusted litter material (500°C, 12 h) to measure AFDM remaining.

We counted macroinvertebrates, identified them to the lowest possible taxonomic level (usually genus), and measured them using ImageJ software (version 1.47; National Institutes of Health, Bethesda, Maryland). We classified taxa to FFGs following Tachet et al. (2010). We calculated individual biomass using published body length–dry mass equations (Dumont et al. 1975, Smock 1980, Meyer 1989, Benke et al. 1999, Baumgärtner and Rothhaupt 2003, Ohta et al. 2011), except Hydracarina, Ostracoda, Cladocera, Oligochaeta, and Nematoda for which we used biovolume data (Ramsay et al. 1997).

Data analysis

All statistical analyses were performed using the programs R (version 2.15.2; R Project for Statistical Computing, Vienna, Austria) and SPSS (version 21.0; IBM Corp., Armonk, New York). We compared riparian canopy cover at the 2 control and 6 fire-affected streams over the first 5 y postfire, and between the fire-affected and control experimental sites from 2003 to 2012 with repeated measures analysis of variance (rmANOVA). We compared mean daily light intensity, mean daily water temperature, and other physicochemical variables between the fire-affected and control sites over the experimental period with 1-way ANOVAs. We log(x)-transformed litter-input data (as g AFDM m^{-2} d^{-1}) and analyzed inputs to fire-affected and control sites with an rmANOVA. We compared the abundances of different macroinvertebrate FFGs in 2 control and 6 fire-affected streams over the first 5 postfire years with rmANOVA. For each sampling date, we used the nonparametric Kruskal–Wallis test to identify the times when macroinvertebrate abundance of different FFGs differed significantly between control and fire-affected sites.

We used an exponential decay model (Bärlocher 2005) to quantify leaf breakdown rates:

$$M_t = M_0 e^{-kt}, \qquad \text{(Eq. 1)}$$

where M_t is the leaf-litter AFDM at time t, M_0 is the initial AFDM corrected for leaching and transportation mass losses, $-k$ is the decomposition rate, and t is the time in d. To test for significant differences in leaf decomposition rates among sites and mesh sizes, we conducted an analysis of covariance (ANCOVA) on ln(x)-transformed AFDM remaining (dependent variable), with site and mesh size as fixed factors and time (d) as a covariate (Zar 2010). We adjusted α levels for pairwise comparisons of decomposition rates between all sites and mesh sizes with Bonferroni corrections. We corrected for temperature effects on leaf breakdown rates, by repeating the analyses using degree days (dd) instead of days (d) as the covariate. We calculated dd as mean daily temperatures accumulated by each sampling day (Minshall et al. 1983, Irons et al. 1994, Menéndez et al. 2003).

We standardized macroinvertebrate abundance and biomass in coarse-mesh leaf bags per gram of leaf-litter AFDM remaining in leaf bags. We log(x + 1)-transformed macroinvertebrate abundance and analyzed differences between sites with ANCOVA, with site as a fixed factor and time as a covariate. We applied Bonferroni corrections to control for comparison-wise error.

RESULTS

Canopy cover and physicochemical measurements

Canopy cover was lower at fire-affected than control sites over the first 5 y postfire ($F_{1,5}$ = 8.2, $p < 0.04$; Fig. 1A) and were consistently lower from 2003–2012 at the fire-affected than control experimental sites ($F_{1,15}$ = 7.1, $p < 0.02$; Fig. 1B). Both sites had alkaline pH, high dissolved

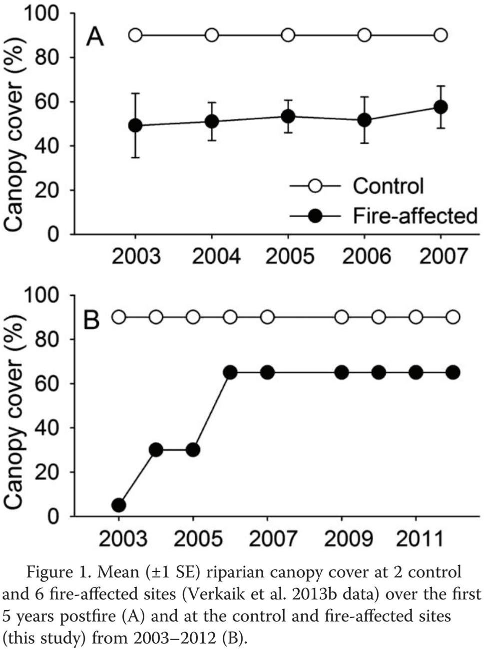


Figure 1. Mean (±1 SE) riparian canopy cover at 2 control and 6 fire-affected sites (Verkaik et al. 2013b data) over the first 5 years postfire (A) and at the control and fire-affected sites (this study) from 2003–2012 (B).

This content downloaded from 23.235.32.0 on Fri, 13 Nov 2015 14:49:38 PM
All use subject to JSTOR Terms and Conditions

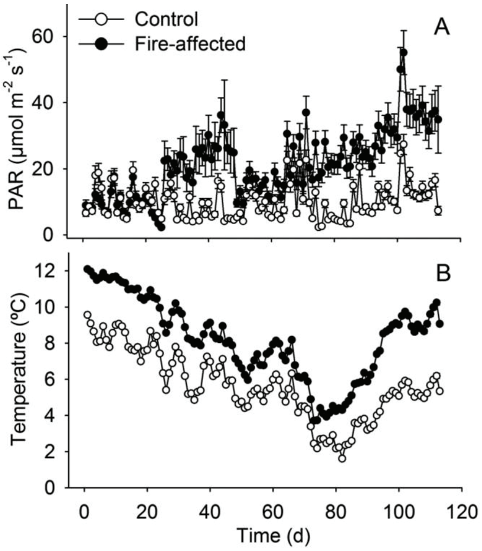


Figure 2. Mean (±1 SE, n = 144 measurements/d) daily light intensity as photosynthetically active radiation (PAR) (A) and water temperature (B) at the control and fire-affected sites during the litter decomposition experiment (23 November 2011–15 March 2012).

O_2 levels, low discharge, and low nutrient concentrations during the study period (Table 1). Mean daily light intensity, mean daily water temperature, and NO_3^--N concentration were substantially higher in the fire-affected than control sites (Table 1, Fig. 2A, B).

Benthic macroinvertebrate communities

The abundances of shredders, scrapers, and gathering collectors peaked in the 1st year postfire at fire-affected sites while remaining relatively constant at control sites, but after >2 y postfire, the abundances of all FFGs were very similar between control and fire-affected sites (Fig. 3A–E). No significant effects of fire on a time × site interaction were detected over the study period (rmANOVA, all $p > 0.05$). In summer 2007, shredders abundances were 523 ± 234 individuals (ind)/sample at the control sites and 417 ± 101 ind/sample at the fire-affected sites. These data indicate that the abundances of FFGs were similar between fire-affected and control sites 3 y before the litter-decomposition experiment and probably at the time of the experiment.

Litter input

Total litter input from October 2011 to October 2012 was 2.5× higher at the control (134.6 ± 19.8 g AFDM

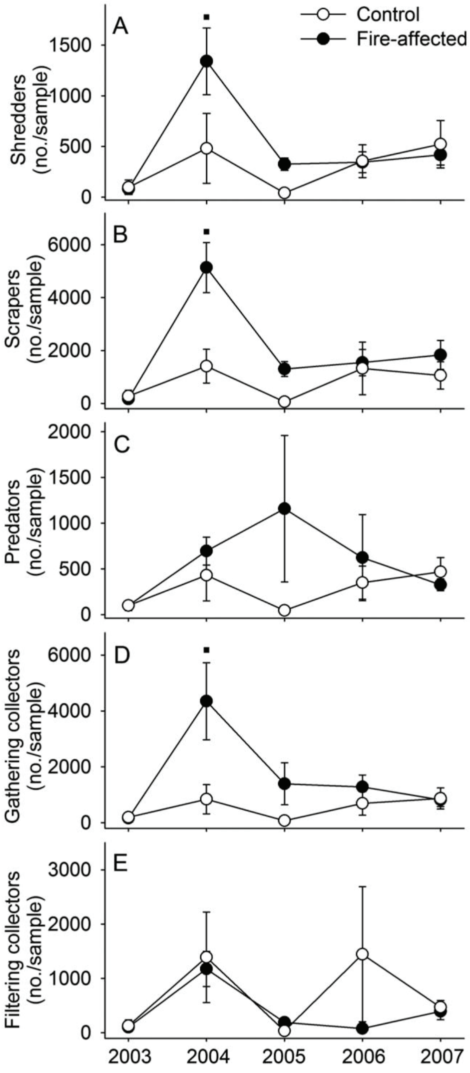


Figure 3. Mean (±1 SE) abundances of shredders (A), scrapers (B), predators (C), gathering collectors (D), and filtering collectors (E) at 2 control and 6 fire-affected sites over the first 5 y postfire (data collected by Verkaik et al. 2013b). ■ indicates marginally significant difference between control and fire-affected sites on a sampling date ($p < 0.10$).

This content downloaded from 23.235.32.0 on Fri, 13 Nov 2015 14:49:38 PM
All use subject to JSTOR Terms and Conditions

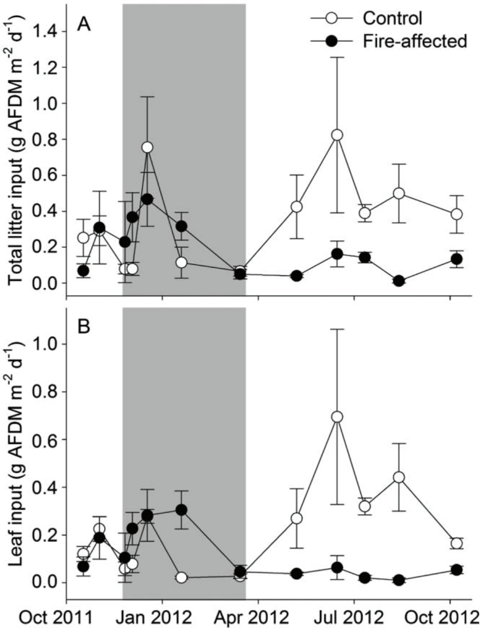


Figure 4. Mean (±1 SE; $n = 3$ replicate litter traps) total litter (A) and leaf (B) inputs at control and fire-affected sites from 4 October 2011 to 4 October 2012. Gray area indicates the time period when the leaf decomposition experiment was done.

m^{-2} y^{-1}) than fire-affected site (52.6 ± 12.0 g AFDM m^{-2} y^{-1}) ($F_{1,4} = 13.2$, $p < 0.025$; Fig. 4A). Leaf inputs were higher at the control than at the fire-affected site ($F_{1,4} = 9.7$, $p < 0.04$; Fig. 4B), but inputs of bark, twigs, fruits or flowers did not differ between sites. Both total litter and leaf inputs were affected by a site × sampling date interaction (total litter: $F_{11,44} = 2.2$, $p < 0.03$; leaf input: $F_{11,44} = 4.5$, $p < 0.001$), with leaf inputs peaking from late spring to early autumn in the control site but remaining at low, constant levels in the fire-affected site (Fig. 4B). Leaf litter made up 62% of the total annual litter input and consisted primarily of leaves of *Q. ilex*, *Q. robur*, *P. alba*, *Viburnum* sp., *Salix* sp., *P. halepensis*, and *Fraxinus* sp.

Leaf-litter decomposition experiment

The loss of poplar leaf mass was well fit by an exponential model, with a slightly better fit to dd than d (R^2 higher in all 4 cases; Table 2). Litter mass loss over time without temperature correction differed between sites ($F_{1,115} = 63$, $p < 0.001$) and mesh sizes ($F_{1,115} = 26$, $p < 0.001$), and was faster at the fire-affected than control site, and in coarse than in fine-mesh bags (Table 2; Fig. 5A). Using dd instead of d as the independent variable, leaf breakdown rates also differed between sites ($F_{1,115} = 10.5$, $p = 0.002$) and mesh sizes ($F_{1,115} = 48$, $p < 0.001$). However, pairwise comparisons showed mass loss differences between sites for coarse- ($F_{1,57} = 19$, $p < 0.001$) but not for fine-mesh bags ($F_{1,57} = 1.9$, $p = 0.18$; Fig. 5B). Litter-loss rates were greater in coarse- than fine-mesh bags at both sites (control: $F_{1,57} = 23$, $p < 0.001$; fire-affected: $F_{1,57} = 30$, $p < 0.001$; Fig. 5B).

Across all coarse-mesh leaf bags, total macroinvertebrate density varied from 1 to 127 and shredder density from 0 to 76 ind/g leaf-litter AFDM. Total macroinvertebrate, shredder, scraper, and predator abundances in coarse-mesh bags were higher at the fire-affected than control site ($F_{1,57} = 9$–16, $p < 0.005$ to < 0.001), but the abundances of gathering and filtering collectors did not differ between sites. The biomass of total macroinvertebrates ($F_{1,57} = 4.8$, $p < 0.04$; Fig. 6A) and shredders ($F_{1,57} = 4.4$, $p < 0.04$; Fig. 6B) in coarse-mesh bags were higher at the fire-affected than control site, but no intersite differences were detected for the biomass of other FFGs (Fig. 6C–F).

DISCUSSION

In a recent review of wildfire effects on Mediterranean streams, Verkaik et al. (2013a) concluded that algal, invertebrate, and amphibian communities recover faster postfire in Mediterranean streams than in streams in other climatic regions (1–4 y in Mediterranean streams vs 5–10 y in non-Mediterranean streams). However, our study indi-

Table 2. Mean (SE) leaf-litter breakdown rates ($-k$) and R^2 values for regressions of ln(x)-transformed leaf mass remaining vs time in days (d) and cumulative degree days (dd).

		d			dd		
Site	Mesh	$-k$	SE	R^2	$-k$	SE	R^2
Control	Coarse	0.00445	0.00025	0.92	0.00084	0.000032	0.96
Control	Fine	0.00370	0.00019	0.93	0.00069	0.000026	0.96
Fire	Coarse	0.00774	0.00036	0.94	0.00098	0.000034	0.97
Fire	Fine	0.00484	0.00038	0.85	0.00062	0.000040	0.90

This content downloaded from 23.235.32.0 on Fri, 13 Nov 2015 14:49:38 PM
All use subject to JSTOR Terms and Conditions

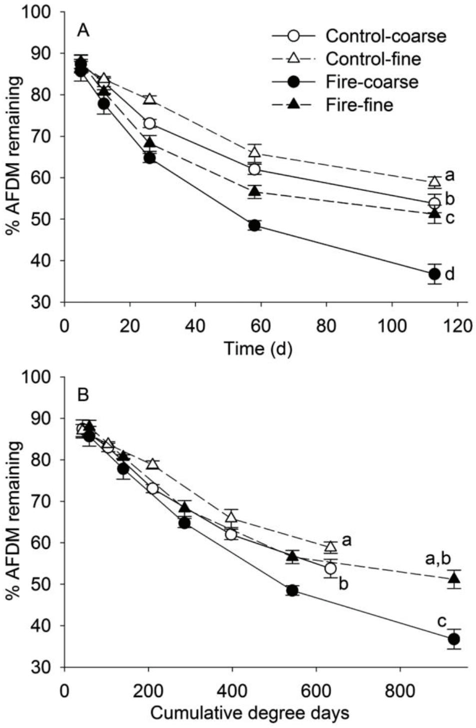


Figure 5. Mean (±1 SE; n = 6 replicate leaf bags) % initial *Populus alba* leaf-litter mass remaining in coarse- and fine-mesh bags at control and fire-affected sites over 113 d expressed over time (A) and over cumulative degree days (dd) (B). Curves with the same letter do not have significantly different decomposition rates ($p > 0.05$).

cates that the legacy effects of wildfire accelerated leaf-litter breakdown in a Mediterranean stream for up to 8 y postfire. We also found that wildfire reduced and altered the timing of allochthonous organic matter inputs into a stream after 8 y postfire. Despite the high resilience to fire of biological communities in Mediterranean streams, our study suggests that past wildfires in Mediterranean streams can have long-term consequences for stream ecosystem processes (Koetsier et al. 2010). The leaf-litter decomposition component of our study was based on only 2 streams (fire-affected vs control), but additional data and observations from these and other sites and times indicate that these 2 streams were similar before fire affected 1 of them and that the proposed mechanisms responsible for our results (see below) are consistent with this information. Thus, we treat our study as an example of how wildfire can trigger long-term changes in ecosystem processes in Mediterranean streams. Our study also illustrates how landscape history, a factor often overlooked in stream ecology, can influence the functioning of lotic ecosystems.

Our results show, as we expected, that opening of the riparian forest canopy by fire increased site light levels and, consequently, water temperature (Gresswell 1999, Koetsier et al. 2010). Wildfire also reduced annual litter inputs, primarily leaf inputs, by 2.5× after 8 y postfire. Leaf inputs peaked from late spring to early autumn in the control site, consistent with the results of Fioretto et al. (2003) for a low Mediterranean shrubland stream and with the contention of Gasith and Resh (1999) and González (2012) that litterfall should be more protracted in Mediterranean than in temperate streams, which have a marked autumnal peak. In contrast, litter inputs to the fire-affected site were lower and more constant, indicating that wildfire affected both the quantity and timing of litter inputs (Jackson et al. 2012).

Leaf-breakdown rates were low but similar to published data for *P. alba* leaves during autumn–winter in Mediterranean rivers (Menéndez et al. 2003). Litter-breakdown rates without temperature correction were faster at the fire-affected than control site in both fine- and coarse-mesh bags. However, fine-mesh decomposition rates did not differ between sites after temperature correction, indicating that microbially mediated decomposition differences between sites were driven almost entirely by differences in temperature. Consistent with our hypothesis, the removal of riparian cover by fire apparently led to higher water temperatures, which increased microbial activity and litter decomposition in the fire-affected site, consistent with literature data indicating the primacy of temperature in determining litter-decomposition rates (Webster and Benfield 1986, Short and Smith 1989, Gessner et al. 1998, Ferreira and Chauvet 2011a). Despite the low nutrient concentrations recorded in our study sites, dissolved NO_3^--N concentrations were >5× higher in the fire-affected than control site, which may have contributed to acceleration of microbial leaf decomposition at the fire-affected site (Benstead et al. 2009, Ferreira and Chauvet 2011b, Woodward et al. 2012). Nevertheless, the lack of difference in decomposition rates in fine-mesh bags between sites when expressed as a function of dd suggests that temperature was the primary factor driving microbial decomposition rates.

Leaf breakdown was faster in coarse- than fine-mesh bags in both sites, and overall decomposition was faster in the fire-affected than the control site. These differences persisted even after temperature corrections. Thus, differences in total decomposition rates between sites should be caused by factors additional to temperature (McArthur et al. 1988). Physical fragmentation and abrasion of leaves were unlikely to be important for leaf breakdown in our study because leaves were deployed in pools at water veloc-

This content downloaded from 23.235.32.0 on Fri, 13 Nov 2015 14:49:38 PM
All use subject to JSTOR Terms and Conditions

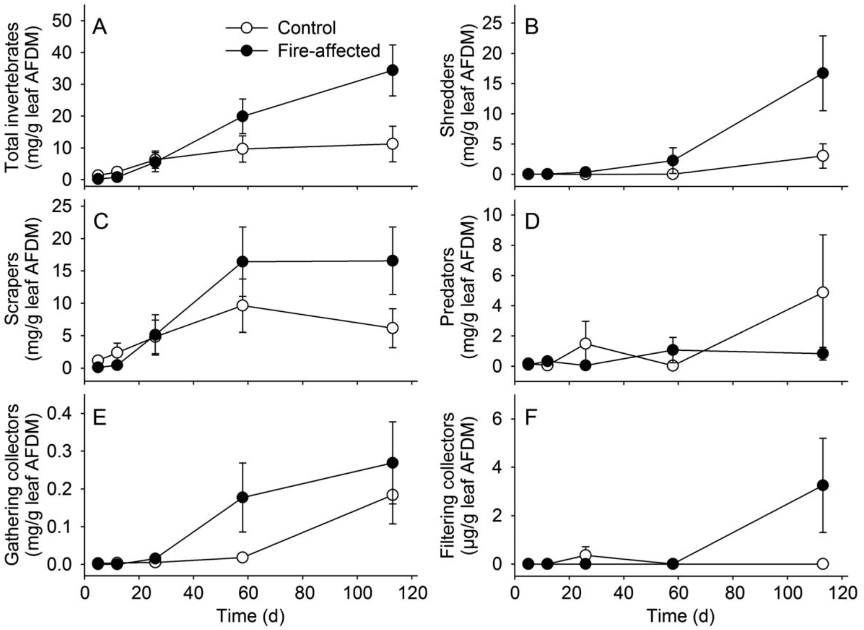


Figure 6. Mean (±1 SE; n = 6 replicate bags) biomass (mg/g remaining leaf ash-free dry mass [AFDM]) of total invertebrates (A), shredders (B), scrapers (C), predators (D), gathering collectors (E), and filtering collectors (F) in coarse-mesh bags at the control and fire-affected streams over the experimental period.

ities <0.15 m/s, far below threshold velocities reported to affect leaf-breakdown rates (Ferreira et al. 2006). Moreover, we saw no evidence of the mechanical fragmentation of leaves. Sediment deposition was unlikely to be important to leaf breakdown because no differences in substrata characteristics were discernible between sites, and burial of leaf bags by sediment was not observed. After dismissing the effects of possible differences in current velocity, mechanical fragmentation, and sediment deposition on leaf decomposition between bags with different mesh sizes, we isolated the effects of shredders on leaf-breakdown rates by subtracting k-values in fine-mesh bags from those in coarse-mesh bags. These calculations showed that shredder-mediated leaf-breakdown rates were 3.9× higher at the fire-affected than the control site (0.0029 vs 0.00075) when expressed /d and 2.4× higher when expressed /dd (0.00036 vs 0.00015). This difference in shredder-mediated leaf-breakdown rates between study sites could be attributed to intersite differences in shredder densities in coarse-mesh bags, which were significantly higher in the fire-affected than the control site by the end of the experiment. Therefore, faster overall leaf decomposition in the fire-affected than the control site probably was caused by both higher microbial activity, driven by higher temperatures, and higher shredder activity in coarse-mesh bags in the fire-affected site.

Higher shredder abundance and biomass in coarse-mesh bags in the fire-affected site were not driven by differences in shredder abundance in the benthos of fire-affected vs control sites because benthic invertebrate abundances recovered quickly postfire, and shredder densities were similar between sites affected and unaffected by fire within 3 y postfire. We also think it is unlikely that shredder aggregation in the leaf bags was driven by refuge responses to predators (Lagrue et al. 2011) because vertebrate predators were absent at both sites and invertebrate predators could move freely into and out of coarse-mesh bags. On the other hand, a variety of studies indicate that shredders aggregate in resource patches when litter availability is low, thereby accelerating litter breakdown (Baldy and Gessner 1997, Rowe and Richardson 2001, Tiegs et al. 2008). We did not measure in-stream CPOM levels, but lower litter inputs to the fire-affected than control sites from May to September probably led to lower in-stream CPOM levels from November to March, when the leaf-bag experiment was done. Our results show that *Populus* leaf-decomposition rates were slow, with leaves lasting several months before

This content downloaded from 23.235.32.0 on Fri, 13 Nov 2015 14:49:38 PM
All use subject to JSTOR Terms and Conditions

being totally skeletonized (e.g., ~54% of the leaf mass remained in control leaf bags after nearly 4 mo in autumn–winter; Fig. 5A, B).

Moreover, wildfires may reduce retention of benthic CPOM in streams by altering the characteristics and inputs of woody debris and removing debris dams via postfire flooding (Vieira et al. 2011, Vaz et al. 2013). A year after a fire in southern California, CPOM levels were lower in fire-affected than reference sites, but in subsequent years, CPOM levels became similar in streams draining burned vs unburned catchments where riparian vegetation remained intact, but remained lower at sites where riparian vegetation burned (Cooper et al. 2015). These results suggest that wildfire effects on CPOM availability depend on the condition of the riparian forest. In addition, higher light availability and NO_3^--N concentration in the fire-affected site could have increased algal growth on leaves, and increased NO_3^--N concentrations could have increased leaf N content, with both enhancing litter quality and attractiveness to shredding invertebrates (Robinson and Gessner 2000, Lagrue et al. 2011, Tant et al. 2013). Therefore, shredder aggregation in leaf bags at the fire-affected site could be attributed to resource tracking by shredders where leaf-litter inputs and probably benthic levels were low and, perhaps, where leaf palatability was high.

The observed fast recovery of shredder abundances postfire despite reductions in litter inputs suggests a weak relationship between litter inputs and shredder density (Linklater 1995), in contrast to studies showing positive relationships between leaf-litter inputs or availability and shredder density (Richardson 1991, Dobson and Hildrew 1992, Boulton and Lake 1992, Friberg 1997, González and Graça 2005). Recovery of shredder abundances after wildfire might be explained by shredder dietary shifts to available secondary resources when leaf litter becomes scarce (Mihuc and Minshall 1995, Costantini and Rossi 1998, Graça 2001, Dangles 2002), such as after a wildfire. Authors of several stable-isotope studies have reported a postfire shift from a detritus-based to a periphyton-based food web in streams (Spencer et al. 2003, Mihuc and Minshall 2005, Cooper et al. 2015). Compared to temperate streams, Mediterranean streams have more macroinvertebrates with traits favoring dispersion and rapid recolonization of disturbed streams, such as aerial active dispersal, more frequent reproduction, and small size, which can explain the fast recovery of macroinvertebrate abundances postfire (Bonada et al. 2007a, Verkaik et al. 2013b). In fact, 5 y postfire, only a few individual taxa with low mobility and limited dispersal capacity were still less abundant in fire-affected than control sites (Verkaik et al. 2013b). The observed peak in the abundances of shredders, scrapers, and gathering collectors in the fire-affected sites in the 1st year postfire may be caused by a short-term increase in resource availability. Britton (1990) reported an increase in leaf-litter input to streams immediately postfire but a decrease over the following years, and Cooper et al. (2015) showed that in-stream algal production where riparian vegetation was burned increased after the 1st postfire wet period apparently because of increased light and high nutrient concentrations, but declined afterward despite the high light levels.

In conclusion, our study shows that a wildfire that occurred 8 y ago accelerated leaf-litter decomposition in a Mediterranean stream because of increased microbial activity associated with higher water temperatures engendered by removal of canopy cover and by shredder aggregation in leaf packs, probably because of scarcity or high palatability of leaf litter in the fire-affected stream. These results indicate that wildfire effects on the riparian forest are key factors mediating wildfire effects on streams, as suggested in previous studies (Arkle and Pilliod 2010, Verkaik et al. 2013a, Cooper et al. 2015). More research on the effects of wildfires on riparian–stream linkages and ecosystem processes is needed to develop effective management practices, given the general lack of scientific studies on these topics and projected increases in wildfire risk.

ACKNOWLEDGEMENTS

We are grateful to Raúl Acosta, Núria Cid, Isabelle Perrée, Iraima Verkaik, and especially to Pau Fortuño for their assistance in the field. We thank Iraima Verkaik for allowing us to include her data to improve our work. We thank the Diputació de Barcelona and the Parc Natural Sant Llorenç del Munt i l'Obac for allowing us to conduct the experiment at their facilities. We thank Meritxell Abril for her comments and suggestions on an earlier version of the manuscript. Thoughtful suggestions and comments by Guest Editor Scott D. Cooper, Editor Pamela Silver, and 3 anonymous referees greatly improved the manuscript. This research was funded by the Ministerio de Economía y Competitividad del Gobierno de España, FURIMED-2 project (ref: CGL2008-03388). PR-L was supported by a FPU (Formación del Profesorado Universitario) fellowship (AP2009-1470) from the Ministerio de Educación, Cultura y Deporte del Gobierno de España.

LITERATURE CITED

Arkle, R. S., and D. S. Pilliod. 2010. Prescribed fires as ecological surrogates for wildfires: a stream and riparian perspective. Forest Ecology and Management 259:893–903.

Baldy, V., and M. O. Gessner. 1997. Towards a budget of leaf litter decomposition in a first-order woodland stream. Comptes Rendus de l'Académie des Sciences - Serie III - Sciences de la Vie 320:747–758.

Bärlocher, F. 2005. Leaf mass loss estimated by litter bag technique. Pages 37–42 *in* M. A. S. Graça, F. Bärlocher, and M. O. Gessner (editors). Methods to study litter decomposition: a practical guide. Springer, Dordrecht, The Netherlands.

This content downloaded from 23.235.32.0 on Fri, 13 Nov 2015 14:49:38 PM
All use subject to JSTOR Terms and Conditions

Baumgärtner, D., and K.-O. Rothhaupt. 2003. Predictive length–dry mass regressions for freshwater invertebrates in a pre-alpine lake littoral. International Review of Hydrobiology 88:453–463.

Benfield, E. F., J. R. Webster, J. L. Tank, and J. J. Hutchens. 2001. Long-term patterns in leaf breakdown in streams in response to watershed logging. International Review of Hydrobiology 86:467–474.

Benke, A. C., A. D. Huryn, L. A. Smock, and J. B. Wallace. 1999. Length–mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. Journal of the North American Benthological Society 18:308–343.

Benstead, J. P., A. D. Rosemond, W. F. Cross, J. B. Wallace, S. L. Eggert, K. Suberkropp, V. Gulis, J. L. Greenwood, and C. J. Tant. 2009. Nutrient enrichment alters storage and fluxes of detritus in a headwater stream ecosystem. Ecology 90:2556–2566.

Betts, E. F., and J. B. Jones. 2009. Impact of wildfire on stream nutrient chemistry and ecosystem metabolism in boreal forest catchments of interior Alaska. Arctic, Antarctic, and Alpine Research 41:407–417.

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

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

Bond, W. J., and J. E. Keeley. 2005. Fire as a global "herbivore": the ecology and evolution of flammable ecosystems. Trends in Ecology and Evolution 20:387–394.

Boulton, A. J., and P. S. Lake. 1992. Benthic organic matter and detritivorous macroinvertebrates in two intermittent streams in south-eastern Australia. Hydrobiologia 241:107–118.

Bowman, D. M. J. S., J. Balch, P. Artaxo, W. J. Bond, M. A. Cochrane, C. M. D'Antonio, R. DeFries, F. H. Johnston, J. E. Keeley, M. A. Krawchuk, C. A. Kull, M. Mack, M. A. Moritz, S. Pyne, C. I. Roos, A. C. Scott, N. S. Sodhi, and T. W. Swetnam. 2011. The human dimension of fire regimes on Earth. Journal of Biogeography 38:2223–2236.

Boyero, L., R. G. Pearson, M. O. Gessner, L. A. Barmuta, V. Ferreira, M. A. S. Graça, D. Dudgeon, A. J. Boulton, M. Callisto, E. Chauvet, J. E. Helson, A. Bruder, R. J. Albariño, C. M. Yule, M. Arunachalam, J. N. Davies, R. Figueroa, A. S. Flecker, A. Ramírez, R. G. Death, T. Iwata, J. M. Mathooko, C. Mathuriau, J. F. Gonçalves, M. S. Moretti, T. Jinggut, S. Lamothe, C. M'Erimba, L. Ratnarajah, M. H. Schindler, J. Castela, L. M. Buria, A. Cornejo, V. D. Villanueva, and D. C. West. 2011. A global experiment suggests climate warming will not accelerate litter decomposition in streams but might reduce carbon sequestration. Ecology Letters 14:289–294.

Britton, D. L. 1990. Fire and the dynamics of allochthonous detritus in a South African mountain stream. Freshwater Biology 24:347–360.

Cooper, S. D., H. M. Page, S. W. Wiseman, K. Klose, D. Bennett, T. Even, S. Sadro, C. E. Nelson, and T. L. Dudley. 2015. Physicochemical and biological responses of streams to wildfire severity in riparian zones. Freshwater Biology. doi:10.1111/fwb.12515

Costantini, M. L., and L. Rossi. 1998. Competition between two aquatic detritivorous isopods–a laboratory study. Hydrobiologia 368:17–27.

Cowling, R. M., F. Ojeda, B. B. Lamont, P. W. Rundel, and R. Lechmere-Oertel. 2005. Rainfall reliability, a neglected factor in explaining convergence and divergence of plant traits in fire-prone mediterranean-climate ecosystems. Global Ecology and Biogeography 14:509–519.

Danger, M., J. Cornut, E. Chauvet, P. Chavez, A. Elger, and A. Lecerf. 2013. Benthic algae stimulate leaf litter decomposition in detritus-based headwater streams: a case of aquatic priming effect? Ecology 94:1604–1613.

Dangles, O. 2002. Functional plasticity of benthic macroinvertebrates: implications for trophic dynamics in acid streams. Canadian Journal of Fisheries and Aquatic Sciences 59:1563–1573.

Dieter, D., D. von Schiller, E. M. García-Roger, M. D. M. Sánchez-Montoya, R. Gómez, J. Mora-Gómez, F. Sangiorgio, J. Gelbrecht, and K. Tockner. 2011. Preconditioning effects of intermittent stream flow on leaf litter decomposition. Aquatic Sciences 73:599–609.

Dobson, M., and A. Hildrew. 1992. A test of resource limitation among shredding detritivores in low order streams in southern England. Journal of Animal Ecology 61:69–77.

Dumont, H. J., I. Van de Velde, and S. Dumont. 1975. The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. Oecologia (Berlin) 19:75–97.

Dury, M., A. Hambuckers, P. Warnant, A. Henrot, E. Favre, M. Ouberdous, and L. François. 2011. Responses of European forest ecosystems to 21st century climate: assessing changes in interannual variability and fire intensity. iForest - Biogeosciences and Forestry 4:82–99.

Earl, S. R., and D. W. Blinn. 2003. Effects of wildfire ash on water chemistry and biota in South-Western U.S.A. streams. Freshwater Biology 48:1015–1030.

Ferreira, V., and E. Chauvet. 2011a. Future increase in temperature more than decrease in litter quality can affect microbial litter decomposition in streams. Oecologia (Berlin) 167:279–291.

Ferreira, V., and E. Chauvet. 2011b. Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. Global Change Biology 17:551–564.

Ferreira, V., M. A. S. Graça, J. L. M. P. de Lima, and R. Gomes. 2006. Role of physical fragmentation and invertebrate activity in the breakdown rate of leaves. Archiv für Hydrobiologie 165:493–513.

Fioretto, A., S. Papa, and A. Fuggi. 2003. Litter-fall and litter decomposition in a low Mediterranean shrubland. Biology and Fertility of Soils 39:37–44.

Friberg, N. 1997. Benthic invertebrate communities in six Danish forest streams: impact of forest type on structure and function. Ecography 20:19–28.

Gama, M., A. L. Gonçalves, V. Ferreira, M. A. S. Graça, and C. Canhoto. 2007. Decomposition of fire exposed Eucalyptus leaves in a Portuguese lowland stream. International Review of Hydrobiology 92:229–241.

This content downloaded from 23.235.32.0 on Fri, 13 Nov 2015 14:49:38 PM
All use subject to JSTOR Terms and Conditions

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

Gessner, M. O., E. Chauvet, and M. Dobson. 1999. A perspective on leaf litter breakdown in streams. Oikos 85:377–384.

Gessner, M. O., C. T. Robinson, and J. V. Ward. 1998. Leaf breakdown in streams of an alpine glacial floodplain: dynamics of fungi and nutrients. Journal of the North American Benthological Society 17:403–419.

González, E. 2012. Seasonal patterns of litterfall in the floodplain forest of a large Mediterranean river. International Review of Hydrobiology 31:173–186.

González, J. M., and M. A. S. Graça. 2005. Influence of detritus on the structure of the invertebrate community in a small Portuguese stream. International Review of Hydrobiology 90:534–545.

Graça, M. A. S. 2001. The role of invertebrates on leaf litter decomposition in streams: a review. International Review of Hydrobiology 86:383–394.

Gresswell, R. E. 1999. Fire and aquatic ecosystems in forested biomes of North America. Transactions of the American Fisheries Society 128:193–221.

IPCC (International Panel on Climate Change). 2014. Climate change 2014: impacts, adaptation, and vulnerability. Part A: global and sectoral aspects. Contribution of Working Group II to the 5th Assessment Report of the Intergovernmental Panel on Climate Change. C. B. Field, V. R. Barros, D. J. Dokken, K. J. Mach, M. D. Mastrandrea, T. E. Bilir, M. Chatterjee, K. L. Ebi, Y. O. Estrada, R. C. Genova, B. Girma, E. S. Kissel, A. N. Levy, S. MacCracken, P. R. Mastrandrea, and L. L. White (editors). Cambridge University Press, Cambridge, UK.

Irons, J. G., M. W. Oswood, R. J. Stout, and C. M. Pringle. 1994. Latitudinal patterns in leaf litter breakdown: is temperature really important? Freshwater Biology 32:401–411.

Jackson, B. K., S. M. P. Sullivan, and R. L. Malison. 2012. Wildfire severity mediates fluxes of plant material and terrestrial invertebrates to mountain streams. Forest Ecology and Management 278:27–34.

Koetsier, P., T. R. B. Krause, and Q. M. Tuckett. 2010. Present effects of past wildfires on leaf litter breakdown in stream ecosystems. Western North American Naturalist 70:164–174.

Lagrue, C., J. S. Kominoski, M. Danger, J.-M. Baudoin, S. Lamothe, D. Lambrigot, and A. Lecerf. 2011. Experimental shading alters leaf litter breakdown in streams of contrasting riparian canopy cover. Freshwater Biology 56:2059–2069.

Lecerf, A., and J. S. Richardson. 2010. Litter decomposition can detect effects of high and moderate levels of forest disturbance on stream condition. Forest Ecology and Management 259:2433–2443.

Linklater, W. 1995. Breakdown and detritivore colonisation of leaves in three New Zealand streams. Hydrobiologia 306:241–250.

Martínez, A., A. Larrañaga, J. Pérez, E. Descals, and J. Pozo. 2014. Temperature affects leaf litter decomposition in low-order forest streams: field and microcosm approaches. FEMS Microbiology Ecology 87:257–267.

McArthur, J V., J. R. Barnes, B. J. Hansen, and L. G. Leff. 1988. Seasonal dynamics of leaf litter breakdown in a Utah alpine stream. Journal of the North American Benthological Society 7:44–50.

Menéndez, M., O. Hernández, and F. A. Comín. 2003. Seasonal comparisons of leaf processing rates in two Mediterranean rivers with different nutrient availability. Hydrobiologia 495:159–169.

Meyer, E. 1989. The relationship between body length parameters and dry mass in running water invertebrates. Archiv für Hydrobiologie 117:191–203.

Mihuc, T. B., and G. W. Minshall. 1995. Trophic generalists vs. trophic specialists: implications for food web dynamics in post-fire streams. Ecology 76:2361–2372.

Mihuc, T. B., and G. W. Minshall. 2005. The trophic basis of reference and post-fire stream food webs 10 years after wildfire in Yellowstone National Park. Aquatic Sciences 67:541–548.

Minshall, G. W., R. C. Petersen, K. W. Cummins, T. L. Bott, J. R. Sedell, C. E. Cushing, and R. L. Vannote. 1983. Interbiome comparison of stream ecosystem dynamics. Ecological Monographs 53:1–25.

Molles, M. C. 1982. Trichopteran communities of streams associated with aspen and conifer forests: long-term structural change. Ecology 63:1–6.

Munné, A., N. Prat, C. Solà, N. Bonada, and M. Rieradevall. 2003. A simple field method for assessing the ecological quality of riparian habitat in rivers and streams: QBR index. Aquatic Conservation: Marine and Freshwater Ecosystems 13:147–163.

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

Ohta, T., Y. Miyake, and T. Hiura. 2011. Light intensity regulates growth and reproduction of a snail grazer (*Gyraulus chinensis*) through changes in the quality and biomass of stream periphyton. Freshwater Biology 56:2260–2271.

Oliver, A. A., M. T. Bogan, D. B. Herbst, and R. A. Dahlgren. 2012. Short-term changes in-stream macroinvertebrate communities following a severe fire in the Lake Tahoe basin, California. Hydrobiologia 694:117–130.

Pancotto, V. A., O. E. Sala, M. Cabello, N. I. López, T. M. Robson, C. L. Ballaré, M. M. Caldwell, and A. L. Scopel. 2003. Solar UV-B decreases decomposition in herbaceous plant litter in Tierra del Fuego, Argentina: potential role of an altered decomposer community. Global Change Biology 9:1465–1474.

Piggott, J. J., K. Lange, C. R. Townsend, and C. D. Matthaei. 2012. Multiple stressors in agricultural streams: a mesocosm study of interactions among raised water temperature, sediment addition and nutrient enrichment. PLoS ONE 7(11): e49873.

Pozo, J., E. González, and J. R. Díez. 1997. Inputs of particulate organic matter to streams with different riparian vegetation. Journal of the North American Benthological Society 16:602–611.

Ramsay, P. M., S. D. Rundle, M. J. Attrill, M. G. Uttley, P. R. Williams, P. S. Elsmere, and A. Abada. 1997. A rapid method for estimating biomass size spectra of benthic metazoan com-

This content downloaded from 23.235.32.0 on Fri, 13 Nov 2015 14:49:38 PM
All use subject to JSTOR Terms and Conditions

munities. Canadian Journal of Fisheries and Aquatic Sciences 54:1716–1724.

Richardson, J. S. 1991. Seasonal food limitation of detritivores in a montane stream: an experimental test. Ecology 72:873–887.

Robinson, C. T., and M. O. Gessner. 2000. Nutrient addition accelerates leaf breakdown in an alpine springbrook. Oecologia (Berlin) 122:258–263.

Robinson, C. T., U. Uehlinger, and G. W. Minshall. 2005. Functional characteristics of wilderness streams twenty years following wildfire. Western North American Naturalist 65:1–10.

Rowe, L., and J. S. Richardson. 2001. Community responses to experimental food depletion: resource tracking by stream invertebrates. Oecologia (Berlin) 129:473–480.

Short, R. A., and S. L. Smith. 1989. Seasonal comparison of leaf processing in a Texas stream. American Midland Naturalist 121:219–224.

Smock, L. A. 1980. Relationships between body size and biomass of aquatic insects. Freshwater Biology 10:375–383.

Spencer, C. N., K. O. Gabel, and F. R. Hauer. 2003. Wildfire effects on stream food webs and nutrient dynamics in Glacier National Park, USA. Forest Ecology and Management 178:141–153.

Tachet, H., P. Richoux, M. Bournard, and P. Usseglio-Polatera. 2010. Invertébrés d'eau douce: systématique, biologie, écologie. CNRS Éditions, Paris, France.

Tant, C. J., A. D. Rosemond, and M. R. First. 2013. Stream nutrient enrichment has a greater effect on coarse than on fine benthic organic matter. Freshwater Science 32:1111–1121.

Tiegs, S. D., F. D. Peter, C. T. Robinson, U. Uehlinger, and M. O. Gessner. 2008. Leaf decomposition and invertebrate colonization responses to manipulated litter quantity in streams. Journal of the North American Benthological Society 27:321–331.

USEPA (US Environmental Protection Agency). 1993. Determination of inorganic ions by ion chromatography. EPA Method 9056. US Environmental Protection Agency, Washington, DC.

Vaz, P. G., E. C. Merten, D. R. Warren, C. T. Robinson, P. Pinto, and F. C. Rego. 2013. Which stream wood becomes functional following wildfires? Ecological Engineering 54:82–89.

Verkaik, I., M. Rieradevall, S. D. Cooper, J. M. Melack, T. L. Dudley, and N. Prat. 2013a. Fire as a disturbance in Mediterranean climate streams. Hydrobiologia 719:353–382.

Verkaik, I., M. Vila-Escalé, M. Rieradevall, and N. Prat. 2013b. Seasonal drought plays a stronger role than wildfire in shaping macroinvertebrate communities of Mediterranean streams. International Review of Hydrobiology 98:1–13.

Vieira, N. K. M., T. R. Barnes, and K. A. Mitchell. 2011. Effects of wildfire and postfire floods on stonefly detritivores of the Pajarito Plateau, New Mexico. Western North American Naturalist 71:257–270.

Vieira, N. K. M., W. H. Clements, L. S. Guevara, and B. F. Jacobs. 2004. Resistance and resilience of stream insect communities to repeated hydrologic disturbances after a wildfire. Freshwater Biology 49:1243–1259.

Vila-Escalé, M. 2009. Efectes d'un incendi forestal en una riera mediterrània (Sant Llorenç del Munt, 2003). PhD Thesis, Universidad de Barcelona, Barcelona, Spain.

Wallace, J. B., S. L. Eggert, J. L. Meyer, and J. R. Webster. 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277:102–104.

Webster, J. R., and E. F. Benfield. 1986. Vascular plant breakdown in freshwater ecosystems. Annual Review of Ecology and Systematics 17:567–594.

Woodward, G., M. O. Gessner, P. S. Giller, V. Gulis, S. Hladyz, A. Lecerf, B. Malmqvist, B. G. McKie, S. D. Tiegs, H. Cariss, M. Dobson, A. Elosegi, V. Ferreira, M. A. S. Graça, T. Fleituch, J. O. Lacoursière, M. Nistorescu, J. Pozo, G. Risnoveanu, M. Schindler, A. Vadineanu, L. B.-M. Vought, and E. Chauvet. 2012. Continental-scale effects of nutrient pollution on stream ecosystem functioning. Science 336:1438–1440.

Young, R. G., C. D. Matthaei, and C. R. Townsend. 2008. Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. Journal of the North American Benthological Society 27:605–625.

Zar, J. H. 2010. Biostatistical analysis. 5th edition. Prentice Hall, Upper Saddle River, New Jersey.

This content downloaded from 23.235.32.0 on Fri, 13 Nov 2015 14:49:38 PM
All use subject to JSTOR Terms and Conditions

PLOS | ONE

RESEARCH ARTICLE

Small but Powerful: Top Predator Local Extinction Affects Ecosystem Structure and Function in an Intermittent Stream

Pablo Rodríguez-Lozano*, Iraima Verkaik, Maria Rieradevall, Narcís Prat

Freshwater Ecology and Management (F.E.M.) Research Group, Departament d'Ecologia, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain

* pablorodriguezlozano@gmail.com

CrossMark
click for updates

Abstract

Top predator loss is a major global problem, with a current trend in biodiversity loss towards high trophic levels that modifies most ecosystems worldwide. Most research in this area is focused on large-bodied predators, despite the high extinction risk of small-bodied freshwater fish that often act as apex consumers. Consequently, it remains unknown if intermittent streams are affected by the consequences of top-predators' extirpations. The aim of our research was to determine how this global problem affects intermittent streams and, in particular, if the loss of a small-bodied top predator (1) leads to a 'mesopredator release', affects primary consumers and changes whole community structures, and (2) triggers a cascade effect modifying the ecosystem function. To address these questions, we studied the top-down effects of a small endangered fish species, *Barbus meridionalis* (the Mediterranean barbel), conducting an enclosure/exclosure mesocosm experiment in an intermittent stream where *B. meridionalis* became locally extinct following a wildfire. We found that top predator absence led to 'mesopredator release', and also to 'prey release' despite intraguild predation, which contrasts with traditional food web theory. In addition, *B. meridionalis* extirpation changed whole macroinvertebrate community composition and increased total macroinvertebrate density. Regarding ecosystem function, periphyton primary production decreased in apex consumer absence. In this study, the apex consumer was functionally irreplaceable; its local extinction led to the loss of an important functional role that resulted in major changes to the ecosystem's structure and function. This study evidences that intermittent streams can be affected by the consequences of apex consumers' extinctions, and that the loss of small-bodied top predators can lead to large ecosystem changes. We recommend the reintroduction of small-bodied apex consumers to systems where they have been extirpated, to restore ecosystem structure and function.

OPEN ACCESS

Citation: Rodríguez-Lozano P, Verkaik I, Rieradevall M, Prat N (2015) Small but Powerful: Top Predator Local Extinction Affects Ecosystem Structure and Function in an Intermittent Stream. PLoS ONE 10(2): e0117630. doi:10.1371/journal.pone.0117630

Academic Editor: John F. Valentine, Dauphin Island Sea Lab, UNITED STATES

Received: July 17, 2014

Accepted: December 29, 2014

Published: February 25, 2015

Copyright: © 2015 Rodríguez-Lozano et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This research was funded by the Ministerio de Economía y Competitividad, Gobierno de España, through the project CGL2008-03388/BOS (FURIMED-2; http://www.mineco.gob.es/portal/site/mineco/). PRL was supported by a FPU contract-fellowship (Formación de Profesorado Universitario) from Ministerio de Educación, Cultura y Deporte, Gobierno de España (ref: FPU2009-1470; http://www.mecd.gob.es/portada-mecd/). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Introduction

Predation is an important species interaction that has implications for biological populations, communities, and ecosystems. In addition to affecting prey abundance and distribution, predation affects other non-prey taxa and ecosystem processes through indirect pathways [1,2]. In recent decades, human activity has caused the extinction of many apex consumers (i.e., top predators) [3,4], and several studies have indicated subsequent ecosystem changes that are complex, unpredictable, and largely unknown [4,5]. Given that current biodiversity loss is biased towards species in the higher trophic levels [3,6], the ecosystem impacts of top-predator decline remain a research priority [7].

The extinction of top predators is often associated with an increase in mesopredators [8–10], i.e., any mid-ranking predator in a food web. An ecosystem may have several mesopredators, and a mesopredator in one system may be a top predator in another system [8]. 'Mesopredator release' often leads to a decrease in the prey [9,10], a straightforward conclusion, termed a 'trophic cascade', when each trophic level is connected in a direct and negative way [9,11,12]. But, as showed in a recent review about apex-mesopredator-prey interactions [10], not all trophic webs have a linear shape. From the 32 studies, Brashares *et al.* [10] found that 40% of the interactions were triangular: those in which top predators feed on mesopredators and also on prey, resulting in intraguild predation (IGP; characterised by predators that feed on other predators with which they share prey taxa). If IGP occurs, the apex consumer exerts top-down control on both mesopredator and prey, and then, apex consumer extinction would liberate mesopredator and prey from its top-down structuring forces. However, in that case, 'mesopredator release' could also lead to an increase on prey top-down control, neutralising apex consumer loss. This would result in a negative or a null net effect on prey taxa, and consequently, dampen the trophic cascade on primary production [13–15]. In addition, according to the predator-mediated coexistence theory [16] and to recent modelling work [17], apex consumer loss can cause secondary extinctions in adjacent and non-adjacent trophic levels [12,18,19], mainly because predators can facilitate coexistence among prey species. Thus, top predator extinctions have been related not only to an increase in mesopredator abundance but also to a decline in biodiversity [9,12].

Intermittent streams are present in all climate areas and are ecologically unique [20,21], but most research in these systems focused on how hydrological variability shapes community attributes and biogeochemical processes [21,22], while the role of top-down structuring forces has been largely overlooked. Furthermore, intermittent streams often lack large aquatic consumers that are often considered to be top predators, and instead, are typically inhabited by predaceous invertebrates and small-bodied fish [23,24]. These systems have been considered a refuge from vertebrate predation [23,25], and even from invertebrate predation, as some studies suggest predatory invertebrates have lower abundances in intermittent than in permanent streams [26]. Other research evidence indicates that predation pressure increases with stream fragmentation in isolated pools, typically in summer, when predatory lentic invertebrates (odonates, hemipterans and coleopterans) replace reophilous taxa [27–30]. Regarding predatory vertebrates, previous studies of intermittent streams show that predatory fish can affect stream macroinvertebrates in terms of: whole community assemblage and total density [31], the densities of specific groups (e.g., air breathing macroinvertebrates [32]), total biomass [33], and prey body condition [34]. Other studies suggest that predatory fish have no effect on macroinvertebrate communities [35]. All these studies were performed in dry season conditions, in isolated pools or in pools that became isolated during the experiment, when predation pressure reaches its peak in these systems. The importance of predation in intermittent streams during periods of flow remains unknown.

The objective of our research was to determine if the loss of an endangered apex consumer from an intermittent stream would result in major changes to ecosystem structure and function. *Barbus meridionalis* (A. Risso, 1827), also known as the Mediterranean barbel, is an endemic small-bodied fish in the Mediterranean intermittent streams of Spain and France, and often act as apex consumer in these ecosystems. This species is considered 'vulnerable' in the Spanish Red Book and 'near threatened' internationally. We studied the top-down impacts of *B. meridionalis* to determine if the loss of the top predator (1) leads to a 'mesopredator release', affecting primary consumers and changing whole community structure, and (2) triggers a cascade effect modifying ecosystem function (i.e., periphyton primary production). *Barbus meridionalis* has been classified as an insectivore benthic species [36] that feeds primarily on chironomid larvae, detritus (which could be explained by its benthic feeding behaviour), mayflies and isopods (mainly primary consumers [37]). Thus, apex consumer extirpation might not lead to 'mesopredator release', and instead could promote a trophic cascade resulting in 'prey release' and lower primary production (i.e., 'prey release' hypothesis, see Fig. 1A). Alternatively, *B. meridionalis* could feed on two trophic levels (i.e., macroinvertebrate secondary and primary consumers), in which case top predator removal would trigger a 'mesopredator release' due to IGP. According to IGP theory, 'mesopredator release' could compensate apex consumer extirpation in terms of prey top-down control, and the trophic cascade would be dampened with no impact on prey or primary production (i.e., 'mesopredator release' hypothesis, see Fig. 1B). To address these questions, we performed a field experiment using enclosure/exclosure mesocosms in a Mediterranean stream where *B. meridionalis* became locally extinct following a wildfire.

Methods

Ethics statements

This study was authorized by the Autonomous Government of Catalonia (Generalitat de Catalunya) and the Natural Parks Department of the Government of Barcelona (Diputació de Barcelona). The University of Barcelona reviewed and approved the project without requirement for ethics approval. Fish were euthanized following the standard protocol recommended by the animal welfare service at the University of Barcelona (anaesthetized using Tricaine methanesulfonate (MS- 222)), and all efforts were made to minimize animal stress and suffering during this study.

Study area

The Vall d'Horta stream (41°40'24'‘N, 2°02'4'‘E; Altitude: 480 m asl) is a first order stream located in the protected area of Sant Llorenç del Munt i l'Obac Natural Park (50 km inland from Barcelona, NE Spain). The main stream course is formed from the confluence of the Pregona and Font del Llor creeks draining to the Ripoll‘s Basin (a tributary of the Besòs River). This hilly area is characterised by a Mediterranean climate and a calcareous geology, with alternating highly permeable and less permeable substrates where springs are located (see [27,38] for a detailed site description). *Barbus meridionalis* is a common fish within these intermittent streams that find refuge in the remaining permanent pools during periods of hydrological disconnection (usually in summer). In August 2003, a wildfire burned a forested area of 4543 ha, affecting 62% of the Vall d'Horta basin. As a consequence of this wildfire, *B. meridionalis* became locally extinct in some of the affected streams, even in the pools, potentially due to chemical changes that occurred during the first rainfall events [39]. The fish population has not recovered since the fire, most likely due to natural and human barriers in the lower part of the study site.

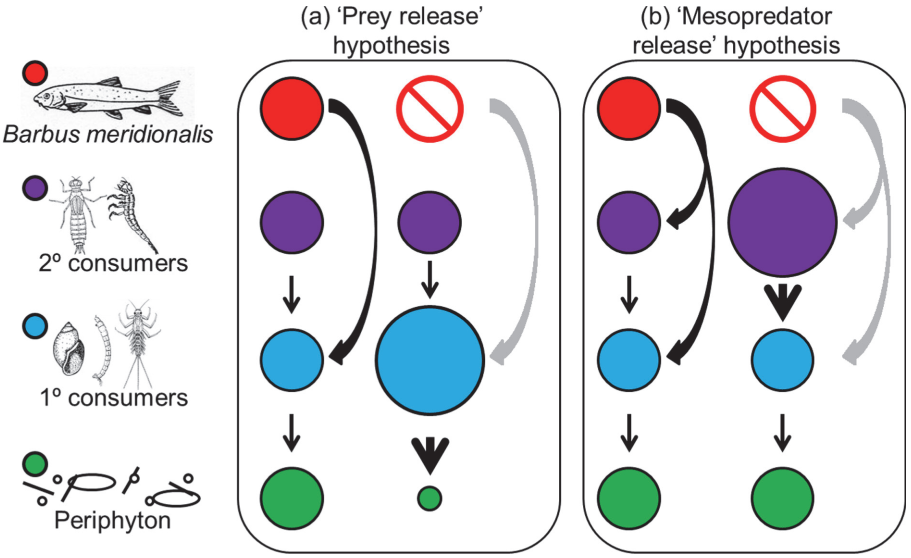


Fig 1. Diagram of the trophic interactions in intermittent stream food webs in the presence and absence of the apex consumer. This diagram describes our two hypotheses related to apex consumer extirpation: a) 'prey release' hypothesis and b) 'mesopredator release' hypothesis. Circumference size in top predator absence diagrams represents the density decrease, increase or persistence compared to the top predator presence diagram. Arrows represent trophic interactions. Thicker arrows = magnified trophic interactions due to apex consumer extirpation; grey arrows = lost trophic interactions after apex consumer extirpation.

doi:10.1371/journal.pone.0117630.g001

We conducted the experiment in a 100 m reach formed by a large pool where riparian vegetation was not burned by the wildfire. This reach was selected because, as observed in the years before the fire, barbels took refuge in these pools to survive periodic drought conditions present in the area when intermittent Mediterranean streams were reduced to isolated pools [40]. Physicochemical water analyses (n = 9) were performed before, during, and at the end of the experiment. The results (presented as the mean ± SE) confirmed that water of this reference stream was hard (conductivity: 520 ± 5 µS cm^{-1}; pH: 7.9 ± 0.1) and oligotrophic ($N\text{-}NO_3^{-1}$: 0.29 ± 0.02 mg l^{-1}; $N\text{-}NH_3$: 0.019 ± 0.003 mg l^{-1}; $P\text{-}PO_4^{3-}$<0.01 mg l^{-1}). The stream discharge averaged 15.7 ± 0.9 l s^1, which, with the very low water velocity in the pool (< 1 cm s^{-1}), naturally kept the pool water renewed and oxygenated (DO_2: 9.6 mg l^{-1}, 84.7%) during our study.

Mesocosm design

We performed an enclosure/exclosure mesocosm experiment to manipulate *B. meridionalis* densities. Removal experiments that simulate the loss of one or more species from a natural community can reveal the consequences of apex consumer extinctions and assess biodiversity-ecosystem function (BD-EF) relationships [41].

We used nine large cages (100 x 100 cm surface, 70 cm height; see Fig. 2) covered with a 10 mm mesh that retained fish but allowed macroinvertebrate emigration/immigration, thereby minimising the impact of our experimental design on the rate of prey exchange with the benthos [42,43]. In each cage, four plastic trays (40 x 40 cm surface, bottom of 1 mm mesh size) were used as replicates (36 trays in total); each tray contained four medium-sized stones for

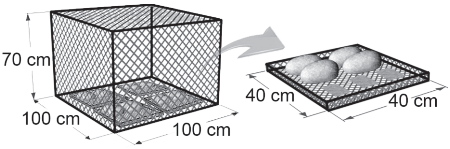


Fig 2. Diagram of the experimental enclosure. Diagram of the experimental enclosure and one of the four identical trays that contained stones for macroinvertebrate colonisation and glass tiles for periphyton colonisation. Dimensions are indicated.

doi:10.1371/journal.pone.0117630.g002

macroinvertebrate colonisation and three glass tiles (2 x 4 cm) for periphyton colonisation (see Fig. 2). Tray substrates within the mesocosms were complex due to the material deposited during the colonisation period; substrate was formed by a mixture of sediment, detritus and leaves, which provided some refuge to invertebrates [44,45] along with the initial added stones. To study the consequences of *B. meridionalis* extirpation, we tested three treatments with varying barbel density levels in the enclosures: i) no fish; ii) barbels at low density (i.e., 2 individuals m^{-2}, the known pre-fire density; A. de Sostoa pers. comm.); and iii) barbels at high density (i.e., 4 individuals m^{-2}, twofold the pre-fire density). Barbels were caught using an electrofishing source downstream from our study site, and individuals selected for the experiment were approximately the same size (total length 101.8 ± 2.6 mm; mean ± SE) and weight (2.3 ± 0.2 g). To ensure similar initial conditions, barbels were kept in observation for 24 h before starting the experiment after electrofishing and transportation.

Sampling and laboratory protocols

The field experiment was conducted in late spring of 2010 before pool disconnection (flow averaged 15.7 ± 0.9 $l\ s^{1}$), over the course of five weeks. Three weeks were allowed for periphyton and macroinvertebrate colonisation, a time previously described as adequate for equilibrating the mesocosm and background macroinvertebrate densities [46]. Two weeks were allowed for barbel interaction. During the colonisation period, the cage tops were opened to facilitate aerial colonisation and the entrance of organic material. Before the addition of barbels to the experimental enclosures, one tray per cage (n = 9) was removed and sampled to test if there were differences in colonisation among cages. Barbel density levels were randomly assigned to enclosures, and the cage tops were closed following barbel introductions to avoid bird or mammal predation. After two weeks of interaction, mesocosms were destructively sampled with the same effort for each tray (n = 27; 9 trays per treatment). Tray contents (with stones) were carefully washed in a 250 μm mesh sieve and preserved in 4% formalin until being processed in the laboratory. All samples were sorted, counted and identified. Taxonomic resolution was primarily to the genus level, including Chironomidae. Some Diptera were identified to the family level, and Oligochaeta, Ostracoda, Cladocera, Copepoda, Hydracarina and terrestrial invertebrates identified to higher levels. Each taxon was categorised as either secondary or primary consumer according to Merritt and Cummins and Tachet *et al.* [47,48]. Periphyton net primary production was measured as the net accumulation of chlorophyll-*a* on artificial substrata [49]. Chlorophyll-*a* was measured after extraction in acetone (90%) for 24 h in the dark at 4°C, sonication for 5 min at 40 kHz, and filtration (GF/F Whatman 0.7 μm-pore size). Following Jeffrey and Humphrey [50], chlorophyll-*a* concentration was determined spectrophotometrically (Perkin-Elmer, Lambda UV/VIS).

In order to test if *B. meridionalis* also feeds on predatory invertebrates (not only on primary consumers), and therefore, if intraguild predation occurs, we analysed barbels' gut contents.

Barbels were euthanised using an overdose of anaesthetic (MS-222). Gut contents were preserved in 4% formalin, sorted, counted, and identified at the same taxonomic level as the benthic samples.

Data analysis

To test differences among the three barbel density treatments, we used the non-parametric Kruskal-Wallis test (K-W test). Then, pairwise Mann-Whitney *U*-tests were used to detect significant differences between treatments. We compared total macroinvertebrate abundance (total number of individuals m^{-2}), taxa richness (number of different taxa), rarefied taxa richness (taxa richness corrected by macroinvetebrate abundance in the sample), Simpson's diversity index (*D*, calculated as $D = \Sigma_i(n_i(n_i - 1)/N(N - 1))$, where n_i is the number of individuals of taxon *i* and *N* is the total number of macroinvertebrates [51]), abundance of common taxa (number of individuals of each abundant taxon, i.e., > 50 ind m^{-2} in the treatment lacking barbels), and periphyton net primary production (net accumulation of chlorophyll-*a*) among the three treatments.

We used permutational multivariate analysis of variance (PERMANOVA, 'Adonis' function in R) on the Bray-Curtis distance matrix, after the log-transformation of the macroinvertebrate abundance data, to test differences in macroinvertebrate community composition among treatments. Afterwards, we used indicator species analysis, using 'IndVal' test in R, to identify which taxa of the macroinvertebrate communities could serve as indicator for each barbel density treatment. The 'IndVal' test calculated the indicator value for each taxon, combining measurements of taxon specificity to each established barbel density treatment with taxon fidelity within each treatment [52]. The significance of 'IndVal' measures was tested using the Monte Carlo test with 9999 permutations.

We also calculated predator:prey ratios for abundance and richness, dividing the abundance (or richness) of secondary consumers by that of primary consumers for each sample. To test for intraguild predation, we also categorised each taxon found in the gut contents as either primary or secondary consumer, and calculated the proportion (%) of each category in the contents. All statistical analyses were performed in R 2.15.2., we used 'vegan' and 'labdsv' packages [53].

Results

We found 81 taxa (76 aquatic invertebrates, 1 amphibian, and 4 terrestrial invertebrates) throughout the mesocosm experiment. Macroinvertebrate communities in the mesocosm were similar to those found during previous research in the stream [38]. Primary consumers were typically chironomids, mayflies (such as *Habroplebia* sp. *Baetis* sp. or *Caenis* sp.), gastropods (such as *Gyraulus* sp. or *Radix* sp.) and crustaceans (Cladocera and Ostracoda); while secondary consumers were dominated by predatory chironomids (*Zavrelimyia* sp. and *Procladius* sp.), water beetles (mainly from Dytiscidae family), hemipterans (*Parasigara* sp.), Odonates (such as *Chalcolestes viridis*, *Sympetrum* sp. or *Aeshna* sp.) and leeches (*Helobdella stagnalis*) (S1 Table). Community-level analyses of the macroinvertebrate samples before the addition of barbels to the enclosures showed a homogeneous colonisation of the experimental cages. Total macroinvertebrate density, taxa richness, Simpson's diversity index, and community composition did not differ among cages (K-W tests, $p>0.1$; Adonis, F = 0.69, p = 0.87). Similarly, significant differences in periphyton net primary production were not observed (K-W test, $\chi^2 = 0.39$, p = 0.83).

Barbel presence reduced macroinvertebrate total density ($\chi^2 = 9.09$, p = 0.011); macroinvertebrate density declined almost by half (46.2%) in the treatment with high barbel density compared to the treatment that did not contain barbels ($U = 12$, $p = 0.01$). We did not detect significant differences among treatments in taxa richness ($\chi^2 = 4.29$, $p = 0.12$) or in the Simpson's diversity index ($\chi^2 = 0.77$, $p = 0.68$). The density of the most abundant macroinvertebrate taxa declined when barbels were present, but vulnerability varied among prey (Fig. 3, S1 Table). We distinguished four patterns of abundance related to barbel density: i) a decrease in abundance proportional to barbel density for some taxa such as *Habrophlebia* sp. and *Chalcolestes viridis* (see Fig. 3C,F); ii) a sharp decrease in abundance at barbel presence (i.e., at both low and high barbel densities but not proportional to barbel presence) for other taxa (e.g., mobile predators *Stictonectes* sp. and *Chaoborus* sp.; see Fig. 3G-H); iii) a significant reduction in taxa abundance only at high barbel density treatment compared to the other treatments (e.g. *Zavrelimyia* sp.; see Fig. 3B); and iv) no change in abundance for other taxa irrespective of barbel densities (e.g., *Gyraulus* sp.; see Fig. 3D).

There were significant differences among the three treatments in the composition of macroinvertebrate communities (Adonis, $F = 2.39$, $p<0.001$). Twelve taxa were identified as indicators in the treatment that did not contain barbels (Table 1) and two taxa in the low barbel density treatment. No indicator taxa were found in the high barbel density treatment.

When we analysed macroinvertebrate communities separately for primary and secondary consumers, we detected that *B. meridionalis* density affected primary consumer abundance ($\chi^2 = 7.38$, $p = 0.025$; Fig. 4A) but not primary consumer richness ($\chi^2 = 1.19$, $p = 0.55$) or rarefied richness ($\chi^2 = 1.42$, $p = 0.49$; Fig. 4B). Top predator absence increased secondary consumer abundance ($\chi^2 = 12.49$, $p = 0.002$; Fig. 4C) and richness before ($\chi^2 = 12.89$, $p = 0.002$) and after rarefaction ($\chi^2 = 8.17$, $p = 0.017$; Fig. 4D). The ratio for predator:prey abundance marginally increased (abundance: $\chi^2 = 5.40$, $p = 0.07$, Fig. 4E) in the absence of barbels, whereas the ratio for predator:prey richness increased significantly (richness: $\chi^2 = 12.00$, $p = 0.002$; rarefied richness: $\chi^2 = 9.92$, $p = 0.007$; Fig. 4F).

Gut content analysis revealed that predatory invertebrates (secondary consumers) amounted to, on average, 22.8 ± 3.5% (mean ± SE) of the individuals in the barbels' gut contents. The most abundant predators found in the gut contents were *Zavrelimyia* sp. (a chironomid), *Parasigara* sp. (an hemipteran), and *Stictonectes* sp. (a water beetle). Other predatory invertebrates including Odonates (such as Lestidae, Libellulidae and Aesnidae families) and other water beetles (such as *Agagus* sp. or *Nebrioporus* sp.) were also found in *B. meridionalis* gut contents.

Periphyton primary production declined in the absence of *B. meridionalis* ($\chi^2 = 17.82$, $p<0.001$; Fig. 5, S1 Table).

Discussion

This study demonstrated that apex consumer extinctions in intermittent streams may result in major changes to the system's structure and function. Like others [8–10,54], our study showed how a top predator extirpation led to 'mesopredator release' in terms of abundance and richness. More importantly, top predator loss led to 'prey release', which contrasts with traditional food web theory and IGP literature. In addition, it triggered a trophic cascade that reduced periphyton primary production. Macroinvertebrate community composition also changed due to *B. meridionalis* absence. These results, along with other studies done in temporary salt marshes [55] and streams [56,57], support that the effects of the loss of small-bodied fish are equivalent to local extinctions of larger apex consumers in other ecosystems (e.g., the arctic fox, wolf, jaguar, sea otter or large reef fish [4,54,58]). Most studies about the consequences of the extinctions of top predators have been focused on large-bodied predators in terrestrial an marine systems [8,9], usually associating large-bodied species to the top of the food webs and small-

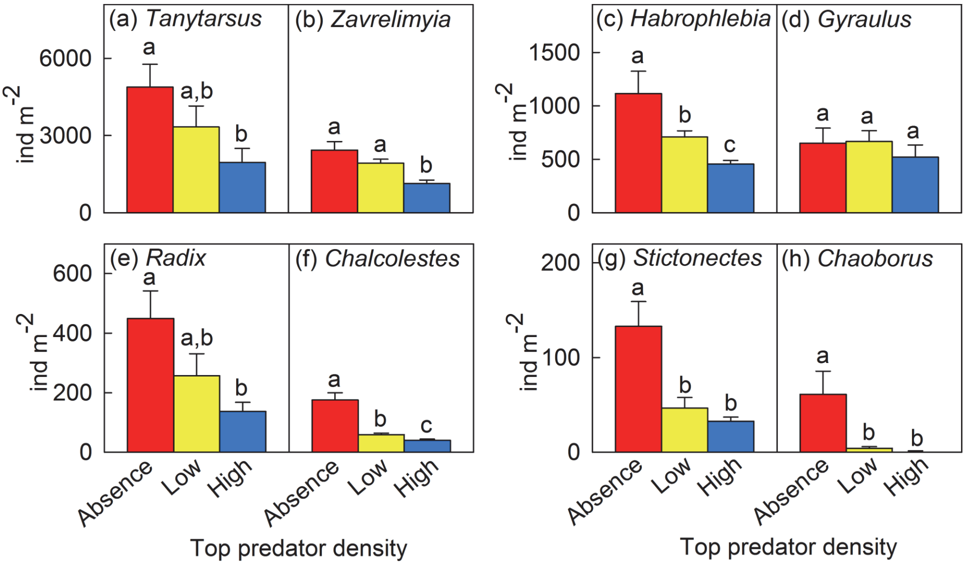


Fig 3. Macroinvertebrate abundance for eight common taxa in the three barbel treatments. Macroinvertebrate abundance for eight of the most abundant taxa (> 50 ind m^{-2} in the treatment lacking barbels) in the three treatments with varying *B. meridionalis* densities. Bars represent mean ± SE (individuals m^{-2}). Graphs are sorted by taxa abundance: (a) *Tanytarsus* sp., (b) *Zavrelimyia* sp., (c) *Habrophlebia* sp., (d) *Gyraulus* sp., (e) *Radix* sp., (f) *Chalcolestes viridis*, (g) *Stictonectes* sp. and (h) *Chaoborus* sp. Red bars = treatment without barbels; yellow bars = treatment with a low density of barbels; blue bars = treatment with a high density of barbels. Different letters correspond to significant differences resulting from the pairwise comparisons among treatments (*U*-test, $p<0.05$).

doi:10.1371/journal.pone.0117630.g003

bodied species to lower trophic levels [5,18], positing also that large-bodied species are at much greater extinction risk than smaller species (see [59]). However, as showed in this study, small-bodied fish in aquatic ecosystems may also exert strong top-down effects, supporting further that 'keystone species' is not a body-size dependent concept, 'keystone species' are those whose effects in the ecosystem are disproportionate to their abundance [60,61]. Moreover, Jenkins [62] suggests that aquatic species, in particular freshwater fish, are more vulnerable to extinction than terrestrial species, and Olden *et al.* [63] highlight that the most globally threatened freshwater fish are small-bodied species. Putting together the results of this study with the fact that numerous small-bodied freshwater fish are at extinction risk, it seems critical to persist in the consideration of the ecological consequences of their possible losses.

Ecosystem structure: 'mesopredator release' and 'prey release'

Mesopredators were more abundant in mesocosms lacking barbels, supporting the 'mesopredator release' hypothesis (see Fig. 1B), which confirms that the loss of small-bodied top predators may have this main common effect with large-bodied predator extirpations [8–10,54]. Several predatory invertebrates that characterised the enclosures lacking barbels (e.g. *Zavrelimyia* sp., *Parasigara* sp. and *Stictonectes* sp.; see Table 1) dominated barbel gut contents, indicating that fish predation contributed to density reduction for these taxa in the presence of barbels. Other taxa, such as *Chaoborus* sp., were not found in barbel gut contents, suggesting that the density decline for some taxa was likely the result of induced emigration.

Table 1. Macroinvertebrate taxa detected as significant indicators for the three barbel density treatments.

Taxa	T	IndVal	P
Chaoborus sp.	1	72.05	<0.001
Cloeon sp.	1	70.88	<0.001
Parasigara sp.	1	69.02	<0.001
Procladius sp.	1	65.10	0.008
Chalcolestes viridis	1	64.04	<0.001
Agabus sp.	1	63.40	0.010
Stictonectes sp.	1	62.69	<0.001
Ostracoda	1	56.53	0.002
Cladocera	1	55.97	0.010
Radix sp.	1	53.33	0.019
Habrophlebia sp.	1	48.90	<0.001
Zavrelimyia sp.	1	44.30	0.012
Oulimnius sp.	2	56.56	0.007
Copepoda	2	49.97	0.021

T—Treatments: 1 = treatment without barbels, 2 = treatment with a low density of barbels. IndVal—indicator value. P—its respective *p*-value.

doi:10.1371/journal.pone.0117630.t001

Mesopredator abundance thus appears to be controlled by the top predator through the combination of predation and possible non-consumption impacts such as competition or induced emigration. Moreover, mesopredator richness also increased in top predator absence. Consequently, a basic element of trophic webs was altered [64]: predator:prey ratios differed among the barbel density treatments (see Fig. 4E-F). Even though predator:prey richness ratio has been previously considered invariant due to underlying community assembly rules [65–67], our results support other studies that did not find conservative predator:prey ratios [68,69] and suggest that secondary and primary consumers respond unequally to the presence of a top predator.

'Mesopredator release' did not lead to a negative or a null effect on primary consumers (see Fig. 4A), which conflicts with the original IGP theory [13–15,70]. In contrast, top predator absence led to increased primary consumer abundance (i.e., 'prey release'), which indicates that the top predator was more effective than mesopredators at suppressing prey. A growing body of literature has posited that top predator presence does not necessarily lead to higher prey abundance if the mesopredator exclusively uses alternate prey [71] or is cannibalistic [72]. However, these new perspectives on IGP are difficult to apply in empirical studies because models continue to oversimplify real food webs (e.g. by modelling food webs with just one intermediate predator). The IGP meta-analysis of Vance-Chalcraft *et al*. [73] concluded that top predator presence usually leads to 'prey release', as predicted by trophic cascade theory, however, it suggested that this is unclear in lotic ecosystems. In this sense, our results showed that the role of the apex consumer was not functionally replaced by the remaining species [74,75], suggesting that the predator assemblage is more important than diversity *per se* [6,76], with species identity being the critical factor.

Our study confirmed top predator extirpation modified the whole community composition. This finding was previously reported for intermittent streams exclusively by Williams *et al*. [31], who found fish have a top-down effect on macroinvertebrate assemblages in isolated pools. But to our knowledge, our study is the first in demonstrating top predator extirpation

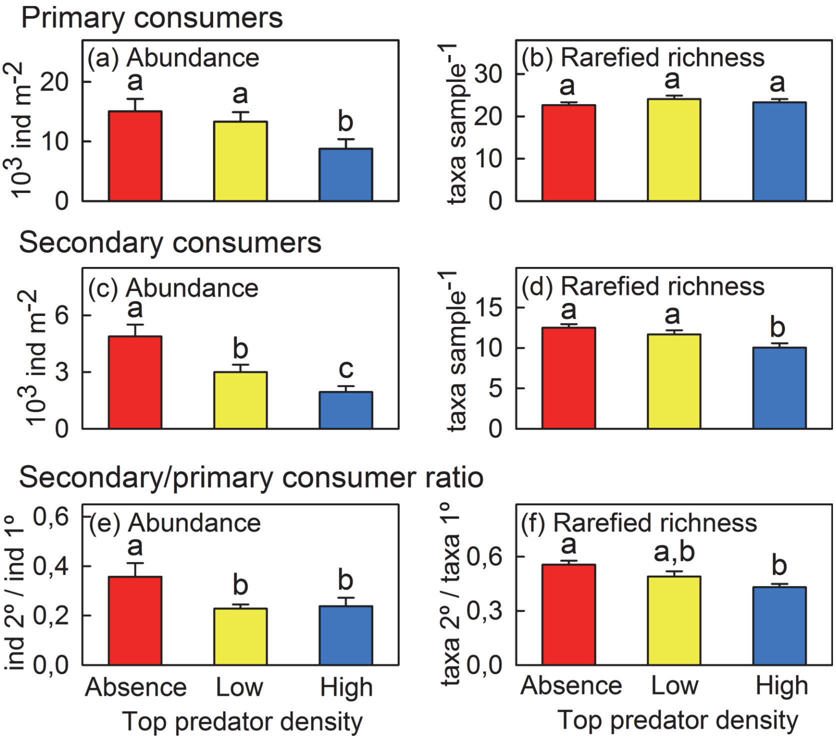


Fig 4. *Barbus meridionalis* density effects on macroinvertebrate abundance and rarefied richness for primary and secondary consumers. *Barbus meridionalis* density effects on macroinvertebrate abundance (mean ± SE individuals m^{-2}) and rarefied taxa richness (mean ± SE rarefied taxa sample^{-1}) for: (a-b) primary consumers, (c-d) secondary consumers, and (e-f) the ratio of secondary to primary consumers (mean ± SE ratio sample^{-1}). Red bars = treatment without barbels; yellow bars = treatment with a low density of barbels; blue bars = treatment with a high density of barbels. Different letters correspond to significant differences resulting from the pairwise comparisons among treatments (*U*-test, $p<0.05$).

doi:10.1371/journal.pone.0117630.g004

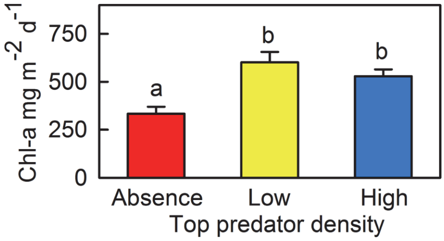


Fig 5. Periphyton net primary production measured as the chlorophyll-*a* on tiles for the three experimental treatments. Bars represent mean ± SE (mg m^{-2} d^{-1}). Red bars = treatment without barbels; yellow bars = treatment with a low density of barbels; blue bars = treatment with a high density of barbels. Different letters correspond to significant differences resulting from the pairwise comparisons among treatments (*U*-test, $p<0.05$).

doi:10.1371/journal.pone.0117630.g005

can change community composition in a running intermittent stream. The treatment lacking barbels was the only that contained a large number of associated indicator taxa (see Table 1). Therefore, the presence of *B. meridionalis* prompted a macroinvertebrate community that was a subset of the macroinvertebrate community without the top predator. The responses of invertebrate populations to barbel presence were highly taxon-dependent, which supports evidence elsewhere that taxa within a trophic level are not functionally equivalent [75,77]. No taxon was however positively affected by barbel presence. We found a statistically significant response even from highly mobile taxa that could rapidly recolonise the enclosures by drift [56,78], indicating a strong top-predator impact. These results indicate that some invertebrates have difficulty co-occurring with this apex consumer. Thus, the local extinction of *B. meridionalis* offered a competitive advantage for these vulnerable species to predation, and did not lead to an extinction cascade, which conflicts with the predator-mediated coexistence theory [16]. Likewise, it contrasts with several studies that relate top predator extinctions to a decline in biodiversity [9,12]; we did not find a relationship between top predator loss and total taxa richness or Simpson's diversity, only for mesopredator richness that increased in top predator absence.

Several studies have emphasised that top predators may be functionally extinct from an ecosystem before being extirpated [18,54,79]. Management efforts to maintain threatened top predators at persistent levels can be ecologically irrelevant if the top predator population does not reach a functionally effective abundance. In our study, the top predator at low density (i.e., pre-fire density) led to an effective suppression of mesopredators, modified the whole macroinvertebrate community composition, and increased indirectly periphyton primary production, compared to the treatment without barbels. However, part of the top predator functional role was only revealed at higher fish density, since the suppression of mesopredator richness and primary consumers' abundance did not occur at low top predator density. These results place apex consumer density as a continuum factor that modulates top predator effects in the ecosystem, confirming that studies about functional extinction thresholds that research top-down effects of apex consumers' extinctions at different densities are particularly relevant for ecosystem restoration and conservation purposes.

Ecosystem function: primary production response

Periphyton net primary production was significantly lower in the absence of *B. meridionalis* (see Fig. 5), confirming a strong trophic cascade effect that modified ecosystem function. This effect could occur through several different mechanisms, which are not necessarily mutually exclusive. Changes in primary consumer density could not fully explain the decline in primary production in top predator absence (see Fig. 4A). However, primary production could be top-down controlled by one or more taxa due to differences in the strength of this interaction, with herbivore identity being the key in the herbivore-producer interface. In this case, *B. meridionalis* extirpation could have increased the abundance of taxa that placed strong pressures on periphyton, triggering a trophic cascade without increasing the total abundance of primary consumers. Another explanation could be that predatory invertebrates were actually omnivorous, and 'mesopredator release' (see Fig. 4C) led to the increased consumption of periphyton. In addition to density-dependent causes, top predator presence could have led to higher primary production through a trait-mediated effect, reducing foraging activity by herbivores [77]. Although positive interactions have been studied less frequently by benthologists [2], *B. meridionalis* presence could have had a direct positive effect on periphyton production via nutrient release and/or by increasing light availability as a result of reduced sediment deposition through feeding foraging movements [35]. These results demonstrate that trophic cascades can be strengthened at the herbivore-producer interface, and contrast with those of Shurin *et al.*

[80], which established that predators more strongly affected primary consumers compared to producers.

Our primary production results have implications for the management of natural and human-altered ecosystems. For instance, our results could modify the general view of how predatory fish abundance is linked to primary production in freshwater ecosystems, given that our results conflicted with traditional trophic cascade theory (which holds that each trophic level is related to the level above and below it in a direct and negative way [11]). In agroecosystems, biological-control practitioners often consider IGP, a very common interaction among aphidophagous predators and parasitoids [14,81]. In this context, Finke and Denno [15] advised against promoting diverse predator assemblages in which IGP was common because it would weaken the suppression of herbivore pests and reduce productivity. These kinds of generalisations can lead to ineffective management practices, particularly given that our results showed that IGP did not dampen the trophic cascade and that neither IGP nor diversity were linked to cascade strength. Instead, in agreement with Borer *et al.* [82], cascade strength depended on the identity of predators and herbivores. Therefore, we recommend that managers place more importance on species identity in decision-making processes to better predict management outcomes.

Conclusions and Implications

We conclude that intermittent streams may be affected by the consequences of top predator extinctions. In this study, the apex consumer was functionally irreplaceable, despite its small-bodied size and even at low population densities, its local extinction led to the loss of an important functional role that resulted in major changes to the ecosystem. Top predator absence triggered a ‘mesopredator release’, but also a ‘prey release’, and changed the whole macroinvertebrate community composition. Regarding ecosystem function, periphyton primary production declined indirectly due to top predator loss. We highlighted that the consequences of this species loss were unforeseen, particularly given that our results were not supported by traditional food web theory. Which ecological responses in mesocosms can be extrapolated to real ecosystems is an open ecological question [83]. Brown et al. [84] demonstrated that aquatic mesocosms can reproduce replicable and realistically not just physicochemistry and macroinvertebrate community composition but complex food webs. Our in-stream mesocosms were carefully design to not be a methodological artefact: mesh size allowed macroinvertebrate emigration/inmigration, and complex tray subsrates within the mesocosms provided refuge to macroinvertebrates. However, spatial complexity and refuge diversity were probably lower in the mesocosms compared to natural stream conditions, which may have increased predator-prey encounter rates. On the other hand, we used conservative top predator densities (i.e., the stream’s pre-fire average density and its double); however, *B. meridionalis* can reach higher densities in stream isolated pools during the dry period (up to 20 ind m^{-2}, usually in summer) suggesting that the impact of this top predator could be even higher than observed here. Thus, despite of the limitations of our study, our main result is consistent, the extirpation of a small-bodied top predator can led to deep system changes in an intermittent stream, at least in the hydrological conditions during our experiment. However, research at larger spatial and temporal scales is needed to integrate the impact of hydrological variability in intermittent streams.

Small-bodied freshwater fish species usually lack commercial value and are often overlooked in conservation management even when considered threatened [85,86]. Based on our results, we recommend that reintroduction programs be considered for small-bodied fish in intermittent streams, where species such *B. meridionalis* had become extirpated. Reintroduction programs would allow not just for recovery of endangered species populations (e.g., *B. meridionalis*), but for the restoration of the ecosystem. Likewise, reintroductions should be considered within a restoration ecology framework, not focusing on mere species presence, but on ecological effectiveness. Because habitat fragmentation often drives apex consumer extirpations [10,87] and can hinder following natural recolonisation, we also recommend the improvement of ecosystem connectivity as a preventive tool as well as a first step in restoration programs. In the context of freshwater ecosystems' conservation, given the high extinction risk of small-bodied freshwater fish, our study evidences that unpredictable ecosystem changes in these ecosystems may occur if conservation efforts are not undertaken.

Supporting Information

S1 Table. Taxa abundance and periphyton primary production data for the three experimental treatments. Taxa are sorted by decreasing abundance in the treatment without top barbels. Category: 1 = primary consumer; 2 = secondary consumer.
(DOCX)

Acknowledgments

We are grateful to F.E.M. research group members and to Alberto Maceda-Veiga, Mario Monroy and Adolf de Sostoa (Animal Biology Department, University of Barcelona) for their field assistance. We thank Antonio García for help with Fig. 2. We would like to thank the *Parc Natural Sant Llorenç del Munt i l'Obac* for allowing us to perform the experiment at their facilities. Thoughtful suggestions and comments by three anonymous reviewers greatly improved the manuscript.

Author Contributions

Conceived and designed the experiments: PRL IV MR NP. Performed the experiments: PRL IV MR NP. Analyzed the data: PRL IV. Contributed reagents/materials/analysis tools: MR NP. Wrote the paper: PRL IV MR NP.

References

1. Gurevitch J, Morrison J, Hedges L (2000) The interaction between competition and predation: a meta-analysis of field experiments. Am Nat 155: 435–453. PMID: 10753073
2. Holomuzki J, Feminella J, Power M (2010) Biotic interactions in freshwater benthic habitats. J North Am Benthol Soc 29: 220–244.
3. Duffy JE (2002) Biodiversity and ecosystem function: the consumer connection. Oikos 99: 201–219.
4. Estes JA, Terborgh J, Brashares JS, Power ME, Berger J, et al. (2011) Trophic downgrading of planet Earth. Science 333: 301–306. doi: 10.1126/science.1205106 PMID: 21764740
5. Marshall KN, Essington TE (2011) Energetic conditions promoting top-down control of prey by predators. PLoS ONE 6(12): e29723. doi: 10.1371/journal.pone.0029723 PMID: 22216348
6. Schneider FD, Brose U (2013) Beyond diversity: how nested predator effects control ecosystem functions. J Anim Ecol 82: 64–71. doi: 10.1111/1365-2656.12010 PMID: 23173560
7. Sutherland WJ, Freckleton RP, Godfray HCJ, Beissinger SR, Benton T, et al. (2013) Identification of 100 fundamental ecological questions. J Ecol 101: 58–67.
8. Prugh LR, Stoner CJ, Epps CW, Bean WT, Ripple WJ, et al. (2009) The rise of the mesopredator. Bioscience 59: 779–791.
9. Ritchie EG, Johnson CN (2009) Predator interactions, mesopredator release and biodiversity conservation. Ecol Lett 12: 982–998. doi: 10.1111/j.1461-0248.2009.01347.x PMID: 19614756
10. Brashares J., Prugh LR, Stoner CJ, Epps CW (2010) Ecological and conservation implications of mesopredator release. In: Terborgh J, Estes JA, editors. Trophic cascades: predators, prey, and the changing dynamics of nature. Washington: Island Press. pp. 221–240.

11. Carpenter SR, Kitchell J, Hodgson J (1985) Cascading trophic interactions and lake productivity. Bioscience 35: 634–639.

12. Henke S, Bryant F (1999) Effects of coyote removal on the faunal community in western Texas. J Wildl Manage 63: 1066–1081.

13. Polis GA, Myers CA, Holt RD (1989) The ecology and evolution of intraguild predation: potential competitors that eat each other. Annu Rev Ecol Syst 20: 297–330.

14. Rosenheim JA, Kaya H, Ehler L, Marois JM, Jaffee BA (1995) Intraguild predation among biological-control agents: theory and evidence. Biol Control 5: 303–335.

15. Finke DL, Denno RF (2005) Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. Ecol Lett 8: 1299–1306.

16. Caswell H (1978) Predator-mediated coexistence: a nonequilibrium model. Am Nat 112: 127–154.

17. Petchey OL, Downing AL, Mittelbach GG, Persson L, Steiner CF, et al. (2004) Species loss and the structure and functioning of multitrophic aquatic systems. Oikos 104: 467–478.

18. Säterberg T, Sellman S, Ebenman B (2013) High frequency of functional extinctions in ecological networks. Nature 499: 468–470. doi: 10.1038/nature12277 PMID: 23831648

19. Borrvall C, Ebenman B (2006) Early onset of secondary extinctions in ecological communities following the loss of top predators. Ecol Lett 9: 435–442. PMID: 16623729

20. Nikolaidis NP, Demetropoulou L, Froebrich J, Jacobs C, Gallart F, et al. (2013) Towards sustainable management of Mediterranean river basins: policy recommendations on management aspects of temporary streams. Water Policy 15: 830–849.

21. Larned ST, Datry T, Arscott DB, Tockner K (2010) Emerging concepts in temporary-river ecology. Freshw Biol 55: 717–738.

22. García-Roger EM, Sánchez-Montoya MDM, Gómez R, Suárez ML, Vidal-Abarca MR, et al. (2011) Do seasonal changes in habitat features influence aquatic macroinvertebrate assemblages in perennial versus temporary Mediterranean streams? Aquat Sci 73: 567–579. PMID: 21178321

23. Meyer JL, Strayer DL, Wallace JB, Eggert SL, Helfman GS, et al. (2007) The contribution of headwater streams to biodiversity in river networks. J Am Water Resour Assoc 43: 86–103.

24. Reich P, McMaster D, Bond N, Metzeling L, Lake PS (2010) Examining the ecological consequences of restoring flow intermittency to artificially perennial lowland streams: patterns and predictions from the Broken—Boosey creek system in Northern Victoria, Australia. River Res Appl 26: 529–545.

25. Williams DD (1996) Environmental constraints in temporary fresh waters and their consequences for the insect fauna. J North Am Benthol Soc 15: 634–650.

26. Bogan MT, Boersma KS, Lytle DA (2013) Flow intermittency alters longitudinal patterns of invertebrate diversity and assemblage composition in an arid-land stream network. Freshw Biol 58: 1016–1028. doi: 10.1007/s10620-012-2434-7 PMID: 23053901

27. Rieradevall M, Bonada N, Prat N (1999) Community structure and water quality in the Mediterranean streams of a natural park (St. Llorenç del Munt, NE Spain). Limnetica 17: 45–56.

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

29. Stanley E, Buschman D, Boulton AJ, Grimm NB, Fisher SG (1994) Invertebrate resistance and resilience to intermittency in a desert stream. Am Midl Nat 131: 288–300.

30. Boulton AJ, Lake PS (1992) The ecology of two intermittent streams in Victoria, Australia. III. Temporal changes in faunal composition. Freshw Biol 27: 123–138.

31. Williams L, Taylor CM, Warren M (2003) Influence of fish predation on assemblage structure of macroinvertebrates in an intermittent stream. Trans Am Fish Soc 132: 120–130.

32. Closs GP (1996) Effects of a predatory fish (Galaxias olidus) on the structure of intermittent stream pool communities in southeast Australia. Aust J Ecol 21: 217–223.

33. Wesner JS (2013) Fish predation alters benthic, but not emerging, insects across whole pools of an intermittent stream. Freshw Sci 32: 438–449.

34. Love JW, Taylor CM, Warren ML (2005) Predator density and dissolved oxygen affect body condition of Stenonema tripunctatum (Ephemeroptera, Heptageniidae) from intermittent streams. Hydrobiologia 543: 113–118.

35. Ludlam JP, Magoulick DD (2010) Environmental conditions and biotic interactions influence ecosystem structure and function in a drying stream. Hydrobiologia 644: 127–137.

36. Benejam L, Angermeier PL, Munné A, García-Berthou E (2010) Assessing effects of water abstraction on fish assemblages in Mediterranean streams. Freshw Biol 55: 628–642.

37. Mas-Martí E, García-Berthou E, Sabater S, Tomanova S, Muñoz I (2010) Comparing fish assemblages and trophic ecology of permanent and intermittent reaches in a Mediterranean stream. Hydrobiologia 657: 167–180.
38. Verkaik I, Vila-Escalé M, Rieradevall M, Prat N (2013) Seasonal drought plays a stronger role than wildfire in shaping macroinvertebrate communities of Mediterranean streams. Int Rev Hydrobiol 98: 1–13.
39. Vila-Escalé M, Vegas-Vilarrúbia T, Prat N (2007) Release of polycyclic aromatic compounds into a Mediterranean creek (Catalonia, NE Spain) after a forest fire. Water Res 41: 2171–2179. PMID: 17397897
40. Vila-Gispert A, Moreno-Amich R (2001) Mass-length relationship of Mediterranean barbel as an indicator of environmental status in South-west European stream ecosystems. J Fish Biol 59: 824–832.
41. Diaz S, Symstad AJ, Stuart Chapin F, Wardle DA, Huenneke LF (2003) Functional diversity revealed by removal experiments. Trends Ecol Evol 18: 140–146.
42. Cooper SD, Walde SJ, Peckarsky BL (1990) Prey exchange rates and the impact of predators on prey populations in streams. Ecology 71: 1503–1514.
43. Allan D, Castillo MM (2007) Stream ecology: structure and function of running waters. Dordrecht: Springer. doi: 10.1093/jxb/erm028 PMID: 25506957
44. Reice S (1991) Effects of detritus loading and fish predation on leafpack breakdown and benthic macroinvertebrates in a woodland stream. J North Am Benthol Soc 10: 42–56.
45. Rosenfeld J (2000) Effects of fish predation in erosional and depositional habitats in a temperate stream. Can J Fish Aquat Sci 57: 1369–1379.
46. Lancaster J, Hildrew A, Townsend CR (1991) Invertebrate predation on patchy and mobile prey in streams. J Anim Ecol 60: 625–641.
47. Merritt R, Cummins KW (1996) An introduction to the aquatic insects of North America. 3rd ed. Duduque: Kendal/Hunt. PMID: 25121335
48. Tachet H, Richoux P, Bournard M, Usseglio-Polatera P (2010) Invertébrés d'eau douce: systématique, biologie, écologie. Paris: CNRS éditions. PMID: 25506974
49. Godwin CM, Carrick HJ (2008) Spatio-temporal variation of periphyton biomass and accumulation in a temperate spring-fed stream. Aquat Ecol 42: 583–595.
50. Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem Physiol Pflanz 167: 191–194.
51. Hurlbert SH (1971) The nonconcept of species diversity: a critique and alternative parameters. Ecology 52: 577–586.
52. Dufrêne M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol Monogr 67: 345–366.
53. R Core Team (2012) R: a language and environment for statistical computing.
54. Beschta RL, Ripple WJ (2009) Large predators and trophic cascades in terrestrial ecosystems of the western United States. Biol Conserv 142: 2401–2414.
55. Compte J, Gascón S, Quintana XD, Boix D (2012) The effects of small fish presence on a species-poor community dominated by omnivores: Example of a size-based trophic cascade. J Exp Mar Bio Ecol 418–419: 1–11.
56. Woodward G, Papantoniou G, Edwards F, Lauridsen RB (2008) Trophic trickles and cascades in a complex food web: impacts of a keystone predator on stream community structure and ecosystem processes. Oikos 117: 683–692.
57. Gido KB, Bertrand KN, Murdock JN, Dodds WK, Whiles MR (2010) Disturbance-mediated effects of fishes on stream ecosystem processes: concepts and results from highly variable prairie streams. Am Fish Soc Symp 73: 593–617.
58. Ripple WJ, Wirsing AJ, Wilmers CC, Letnic M (2013) Widespread mesopredator effects after wolf extirpation. Biol Conserv 160: 70–79.
59. Cardillo M (2003) Biological determinants of extinction risk: why are smaller species less vulnerable? Anim Conserv 6: 63–69.
60. Paine R (1969) A note on trophic complexity and community stability. Am Nat 103: 91–93.
61. Kotliar N, Baker B, Whicker A, Plumb G (1999) A critical review of assumptions about the prairie dog as a keystone species. Environ Manage 24: 177–192. PMID: 10384028
62. Jenkins M (2003) Prospects for biodiversity. Science 302: 1175–1177. PMID: 14615529
63. Olden JD, Hogan ZS, Vander Zanden (2007) Small fish, big fish, red fish, blue fish: size-biased extinction risk of the world's freshwater and marine fishes. Glob Ecol Biogeogr 16: 694–701.

64. Elton CS (1927) Animal Ecology. London: Sidgwick and Jackson. PMID: 20773612

65. Cohen JE (1977) Ratio of prey to predators in community food webs. Nature 270: 165–167.

66. Warren PH, Gaston KJ (1992) Predator-prey ratios: a special case of a general pattern? Philos Trans R Soc B Biol Sci 338: 113–130.

67. Jeffries MJ (2002) Evidence for individualistic species assembly creating convergent predator:prey ratios among pond invertebrate communities. J Anim Ecol 71: 173–184.

68. Wilson JB (1996) The myth of constant predator:prey ratios. Oecologia 106: 272–276.

69. Shulman RS, Chase JM (2007) Increasing isolation reduces predator:prey species richness ratios in aquatic food webs. Oikos 116: 1581–1587.

70. Snyder WE, Ives AR (2001) Generalist predators disrupt biological control by a specialist parasitoid. Ecology 82: 705–716.

71. Holt RD, Huxel GR (2007) Alternative prey and the dynamics of intraguild predation: theoretical perspectives. Ecology 88: 2706–2712. PMID: 18051637

72. Rudolf V (2007) The interaction of cannibalism and omnivory: consequences for community dynamics. Ecology 88: 2697–2705. PMID: 18051636

73. Vance-Chalcraft HD, Rosenheim JA, Vonesh JR, Osenberg CW, Sih A (2007) The influence of intraguild predation on prey suppression and prey release: a meta-analysis. Ecology 88: 2689–2696. PMID: 18051635

74. Ernest SK, Brown JH (2001) Delayed compensation for missing keystone species by colonization. Science 292: 101–104. PMID: 11292873

75. Chalcraft DR, Resetarits WJ (2003) Predator identity and ecological impacts: functional redundancy or functional diversity? Ecology 84: 2407–2418.

76. Cardinale BJ, Srivastava DS, Duffy JE, Wright JP, Downing AL, et al. (2006) Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature 443: 989–992. PMID: 17066035

77. Schmitz OJ, Krivan V, Ovadia O (2004) Trophic cascades: the primacy of trait-mediated indirect interactions. Ecol Lett 7: 153–163.

78. Gilliam J, Fraser DF, Sabat AM (1989) Strong effects of foraging minnows on a stream benthic invertebrate community. Ecology 70: 445–452.

79. Soulé ME, Estes JA, Berger J, Martinez del Rio C (2003) Ecological effectiveness: conservation goals for interactive species. Conserv Biol 17: 1238–1250.

80. Shurin JB, Borer ET, Seabloom EW, Anderson K, Blanchette CA, et al. (2002) A cross-ecosystem comparison of the strength of trophic cascades. Ecol Lett 5: 785–791.

81. Gagnon A, Heimpel G, Brodeur J (2011) The ubiquity of intraguild predation among predatory arthropods. PLoS ONE 6(11): e28061. doi: 10.1371/journal.pone.0028061 PMID: 22132211

82. Borer E, Seabloom E, Shurin JB, Anderson K, Blanchette CA, et al. (2005) What determines the strength of a trophic cascade? Ecology 86: 528–537.

83. Lamberti G, Steinman A (1993) Research in artificial streams: applications, uses, and abuses. J North Am Benthol Soc 12: 313–384.

84. Brown LE, Edwards FK, Milner AM, Woodward G, Ledger ME (2011) Food web complexity and allometric scaling relationships in stream mesocosms: implications for experimentation. J Anim Ecol 80: 884–895. doi: 10.1111/j.1365-2656.2011.01814.x PMID: 21418207

85. Saddlier S, Koehn JD, Hammer MP (2013) Let's not forget the small fishes—conservation of two threatened species of pygmy perch in south-eastern Australia. Mar Freshw Res 64: 874–886.

86. Williams DD (2006) The biology of temporary waters. New York: Oxford University Press. PMID: 25590126

87. Staddon P, Lindo Z, Crittenden PD, Gilbert F, Gonzalez A (2010) Connectivity, non-random extinction and ecosystem function in experimental metacommunities. Ecol Lett 13: 543–552. doi: 10.1111/j.1461-0248.2010.01450.x PMID: 20236160