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PATRONES DE BIODIVERSIDAD FLUVIAL A GRAN ESCALA Y EFECTOS DE LAS PRESAS EN LOS RÍOS ESPAÑOLES

PhD THESIS

LARGE-SCALE RIVERINE BIODIVERSITY PATTERNS AND DAM EFFECTS ON SPANISH RIVERS

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La alteración hidrológica provocada por presas, el cambio climático y cambios en el uso del suelo producen efectos generalizados en los ecosistemas de agua dulce y ponen en riesgo servicios ecosistémicos esenciales. Los ecosistemas fluviales son especialmente vulnerables a estos factores de estrés, con tasas de pérdida de biodiversidad alarmantes. En este contexto, el objetivo principal de esta tesis doctoral es explorar los patrones espaciales de biodiversidad en los ríos españoles y cómo las presas y embalses los modifican. Utilizando taxonomía y rasgos de diatomeas, macroinvertebrados y peces, evaluamos el papel de los gradientes ambientales y procesos relacionados con la dispersión en la estructura de las comunidades biológicas en ríos prístinos de toda España. En ríos afectados por presas de regadío e hidroeléctricas en el norte del país, investigamos las respuestas ecológicas en zonas de montaña y en un diseño de control-impacto. Las conclusiones del estudio aportan una perspectiva útil para el desarrollo de estrategias de conservación eficaces dirigidos a los impulsores del cambio global y adaptados a los diferentes esquemas operativos de las presas.

Hydrological alteration caused by dams, changing climates, and land use-cover changes produce pervasive effects on freshwater ecosystems and undermine the delivery of essential ecosystem services. Riverine ecosystems are particularly vulnerable to these stressors, with alarming biodiversity loss rates. In this context, the primary goal of this PhD thesis is to explore spatial biodiversity patterns in Spanish rivers and how dams and reservoirs modify them. Using diatom, macroinvertebrate, and fish taxonomic and trait-based communities, we evaluated the role of key environmental gradients and dispersal-related processes in structuring biological communities in near-pristine streams throughout Spain. In streams impacted by irrigation and hydropower dams in the north of the country, we investigated the ecological responses in mountain areas and in a control-impact design. The study's findings provide useful insights into the development of effective conservation efforts directed towards global change drivers and the different dams' operational schemes.

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Resumen

De acuerdo con la Normativa de Gestión Académica de Estudios de Doctorado regulados por el Real Decreto 99/2011 en relación a los requerimientos exigidos para aquellas Tesis redactadas en una lengua distinta a la castellana, a continuación se presenta un resumen global en español del documento original redactado en inglés.

Introducción y antecedentes de la investigación

Aguas dulces de la superficie terrestre

El agua dulce representa sólo el 0,01 % de todo el agua disponible en la Tierra (Lehner and Döll, 2004). A pesar de ello, sus ecosistemas albergan un porcentaje desproporcionadamente alto de la biodiversidad del planeta: alrededor del 10 % de todas las especies conocidas, incluyendo un tercio de todos los vertebrados (Strayer and Dudgeon, 2010). Los ecosistemas de aguadulce también proporcionan una serie de servicios ecosistémicos esenciales, como la provisión de agua para consumo y riego, el suministro de alimentos (p.e., pescado), la dotación valores culturales (ocio, ecoturismo, religiosidad), y contribuyen en otros servicios como la formación del suelo, el ciclo de nutrientes y el suministro de hábitats (Durance et al., 2016; Petsch et al., 2022).

Sin embargo, en la gestión de estos ecosistemas a menudo se ha priorizado, en muchas ocasiones, aquellos servicios que generan beneficios macroeconómicos en detrimento de los hábitats acuáticos, su flora, su fauna y otros beneficios que ofrecen a las sociedades (Tickner et al., 2020b). Alrededor del 65 % de los hábitats acuáticos sustentados por agua dulce están sometidos a múltiples factores de estrés derivados, principalmente, de actividades antropogénicas (WWAP, 2019), como el cambio climático, la regulación de los caudales causada por las presas, la contaminación del agua y/o la introducción y proliferación de especies invasoras (Dudgeon, 2019; Reid et al., 2019; Vörösmarty et al., 2010).

Estado actual de los sistemas de agua dulce

Los profundos cambios geológicos, sociológicos, antropológicos y ecológicos de la historia reciente de la Tierra que engloban el "Antropoceno" han provocado impactos abrumadores en los ecosistemas, siendo especialmente relevantes en los ecosistemas dulceacuícolas (Heino et al., 2021; Waters et al., 2016) y, consecuentemente, la escala

actual de pérdida de biodiversidad en estos ecosistemas es alarmante. El Índice Planeta Vivo muestra una importante disminución de las poblaciones de agua dulce (equivalente a un 4 % anual desde 1970), especialmente en anfibios, reptiles y peces en todas las regiones (Baker et al., 2020). Los humedales han perdido aproximadamente el 35 % de su superficie desde la década de los 70s, lo que equivale a tres veces la tasa de pérdida de bosques y ha supuesto la disminución de las poblaciones del 81 % de las especies típicas de estos sistemas (Gardner and Finlayson, 2018).

La sobreexplotación de los recursos hídricos, la contaminación del agua, la alteración de los caudales y la invasión de especies exóticas son algunas de las principales causas de la pérdida de biodiversidad en ecosistemas de agua dulce (Dudgeon, 2019; Dudgeon et al., 2006; Reid et al., 2019). No obstante, inversiones destinadas a la conservación de los ecosistemas de agua dulce han sido, por lo general, menores que en sus homólogos terrestres, lo que contrasta con el riesgo que presentan (Abell et al., 2011). Los ecosistemas de agua dulce necesitan urgentemente estrategias y acciones de gestión eficaces para detener la pérdida de biodiversidad. Su grado de eficacia dependerá de la capacidad de preservar la configuración natural del hábitat junto con la dinámica de las poblaciones, así como de un conocimiento profundo de los principales factores que rigen los patrones de distribución de la biota (Allan and Flecker, 1993; He et al., 2018; Olden et al., 2010; Tickner et al., 2020b).

Desde el desarrollo de los programas de cambio global en la década de 1980 y la Conferencia de Dublín sobre el Agua en 1992, la preocupación relacionada con el agua ha sido debatido en las agendas internacionales de sostenibilidad. Algunos de los Objetivos de Desarrollo Sostenible (ODS) de las Naciones Unidas implantados en 2015 estaban dirigidos a la protección y restauración de los ecosistemas acuáticos, y el Convenio sobre la Diversidad Biológica (CDB) adoptó el Plan Estratégico para la Diversidad Biológica 2011-2020 y las Metas de Aichi 2020. Sin embargo, en comparación con las zonas terrestres, costeras y marinas, estas acciones globales de conservación y restauración no contemplan con profundidad las aguas continentales, y solo se priorizan los servicios ecosistémicos de agua dulce más inmediatos (Gonçalves and Hermoso, 2022). Por ello, los científicos demandan una mayor atención sobre los ecosistemas de agua dulce en el Marco Global de Biodiversidad Post-2020, con acciones de conservación y restauración que prioricen estos ecosistemas (GEO BON and FWBON, 2022).

Tickner et al. (2020b) propusieron una estrategia de emergencia para invertir el declive de la biodiversidad de los ecosistemas de agua dulce y orientar los futuros programas y acciones globales en materia de biodiversidad. Este plan de emergencia se estructura en torno a las principales causas de pérdida de biodiversidad de agua dulce identificadas por Dudgeon et al. (2006) y prioriza acciones como la implantación

de caudales ecológicos, la mejora de la calidad del agua, la protección y restauración de hábitats críticos y la conectividad de agua dulce.

Estado actual de los ecosistemas de agua dulce en Europa

La aprobación de la Directiva Marco del Agua (DMA) de la Unión Europea (European Commission, 2000) en 2000 marcó un hito en la política de aguas de los Estados miembros. La DMA estableció un marco de actuación para la restauración de los ecosistemas acuáticos con el fin último de alcanzar un buen estado ecológico de todos los ríos, lagos y humedales europeos.

La DMA cambió el paradigma de la gestión del agua de una perspectiva antropocéntrica a una ecocéntrica, y el agua pasó a considerarse como el soporte de numerosos ecosistema en lugar de como un recurso a explotar por las sociedades humanas (European Commission, 2000; Santos et al., 2021). El estado ecológico de las masas de agua empezó a evaluarse desde una perspectiva biológica, en lugar de sólo por sus propiedades fisicoquímicas, desplazando los objetivos de gestión del control de la contaminación a la integridad del ecosistema en su conjunto (Hering et al., 2010).

Tras el primer ciclo de gestión (2010-2015), que se fijó inicialmente como plazo para alcanzar los principales objetivos de la DMA, los planes hidrológicos de cuenca indicaron que alrededor del 60 % de las aguas superficiales en Europa no alcanzaban el buen estado ecológico. En general, los ríos y las aguas de transición obtuvieron puntuaciones más bajas que las aguas costeras y los lagos (European Environment Agency, 2018a), con un predominio masas de agua en mal estado en Europa central, mientras que na alta proporción de masas de agua del norte de Escandinavia, Escocia, Estonia, Rumanía, Eslovaquia y la región mediterránea se encontraba en buen estado ecológico (Figura 1). En este punto, se concedió a los Estados miembros la posibilidad de alcanzar el buen estado ecológico de sus masas de agua en 2021 o, a más tardar, en 2027 (Ramos et al., 2018).

En comparación con 2009, el informe presentado en 2015 que alrededor del 20 % de las masas de agua superficiales (es decir, 16.000 masas de agua) mostraban cierto progreso hacia un buen estado ecológico (European Commission, 2019a). En muchos casos, se detectaron mejoras en elementos de calidad de forma independiente (es decir, indicadores fisicoquímicos, hidromorfológicos o biológicos), sin embargo, no reflejaron una mejora del estado ecológico general debido al principio del "*uno fuera, todos fuera*" (es decir, la puntuación más baja de cualquier elemento de calidad determina el estado ecológico general de la masa de agua; European Commission, 2019a; Santos et al., 2021).

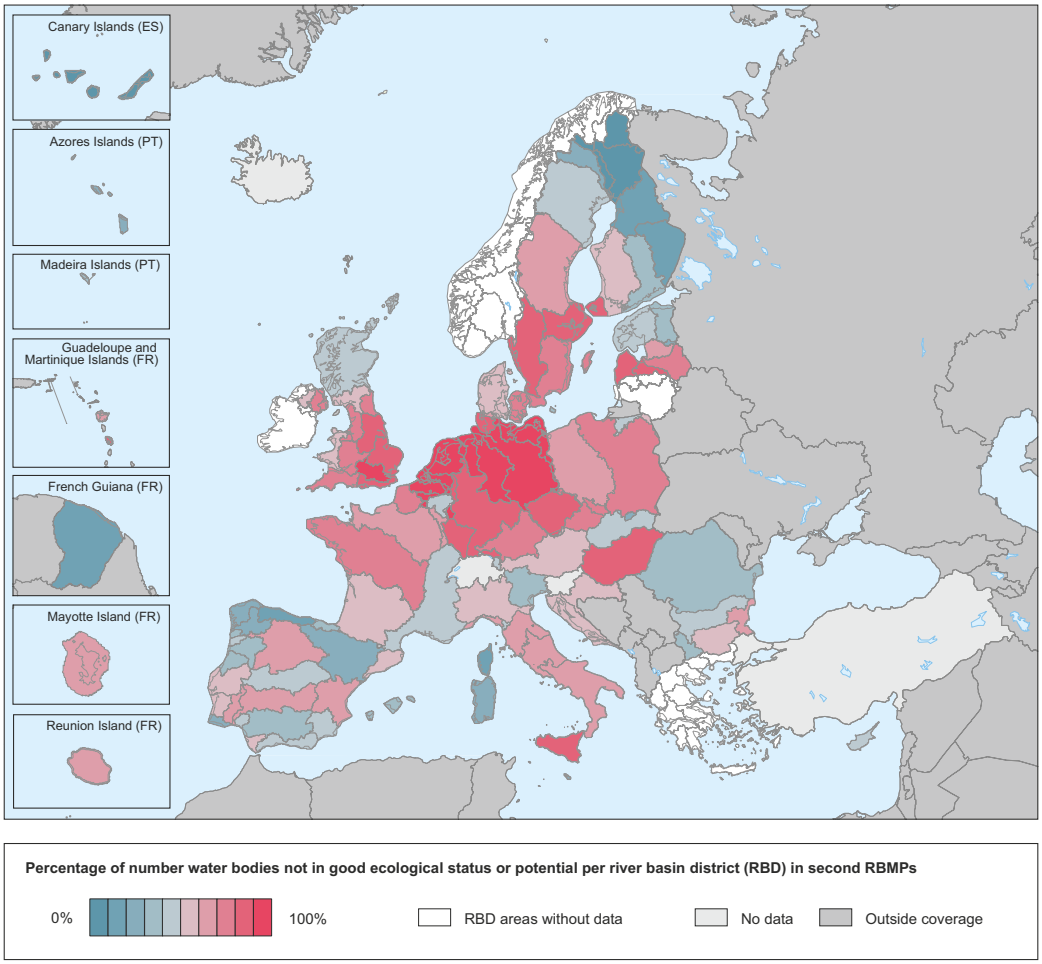


Figura 1 Porcentaje de masas de agua de los Estados miembros de la UE que no alcanzaron el buen estado ecológico en el segundo ciclo de planes hidrológicos de cuenca (European Commission, 2019a).

Aunque los Estados miembros no consiguieron aún alcanzar el objetivo principal de la DMA, ésta permitió ahondar en el conocimiento sobre los recursos hídricos y los ecosistemas acuáticos en todo Europa. El número de masas de agua evaluadas aumentó progresivamente, así como la confianza general en la evaluación del estado ecológico (European Environment Agency, 2018a). Además, la DMA promovió el desarrollo y la aplicación de metodologías de evaluación ecológica comparables en toda Europa, mejorando considerablemente la eficacia de los programas de seguimiento (Birk et al., 2012; Carvalho et al., 2019).

El uso del agua en el mundo

El consumo mundial de agua se ha multiplicado por seis en el último siglo, con un incremento anual del 1,8 % (Boretti and Rosa, 2019; Wada et al., 2016). Este aumento puede atribuirse a una combinación de crecimiento demográfico, desarrollo

económico y cambios en los patrones de consumo de agua (Flörke et al., 2013; United Nations, 2021).

El sector agrícola es el principal consumidor de agua, representando el 69 % del consumo mundial y alcanzando hasta el 95 % en algunos países en desarrollo (FAO, 2011). La industria, la energía y la generación de electricidad representan el 19 % del consumo mundial de agua, mientras que el 12 % restante corresponde al uso doméstico del agua (Figura 2).

Las previsiones indican que la demanda mundial aumentará considerablemente en las próximas décadas, sobre todo los usos doméstico e industrial en las economías emergentes y en los países de ingresos medios y bajos (Ritchie and Roser, 2017; United Nations, 2021). Por tanto, es esencial incremental y asegurar la disponibilidad de agua (p.e., a través de procesos de desalinización del agua salada o la construcción de embalses) y reducir su consumo (p.e., mediante el usos de métodos de riego eficientes o promoviendo el reciclaje del agua; He et al., 2021).

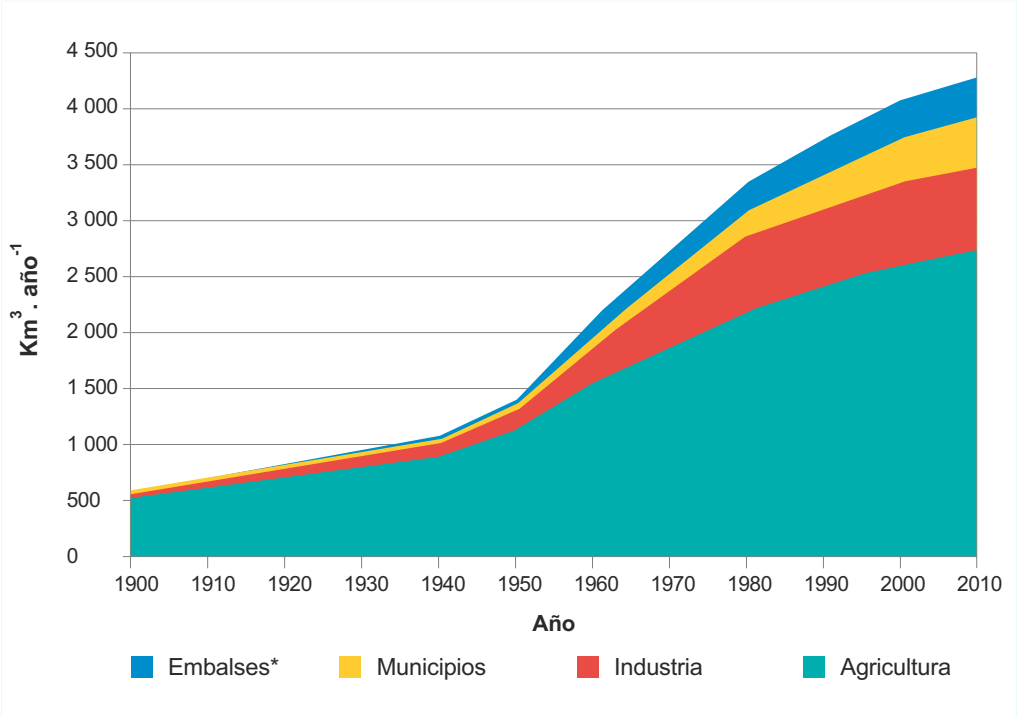


Figura 2 Consumo mundial de agua entre 1900 y 2010 (AQUASTAT, 2010; United Nations, 2021). *Los embalses se refieren a la evaporación de los lagos artificiales.

El uso del agua en Europa

En Europa, el principal usuario de agua es la agricultura (58 %), seguida del agua de refrigeración para la producción de energía (18 %), la industrias manufactureras, mineras y de la construcción (14 %) y el uso doméstico (10 %; European Environment

Agency, 2021). Sin embargo, este patrón general varía entre los Estados miembros: en los países del norte, la industria y la producción de electricidad destacan como los principales consumidores de agua (67 % en 2017), mientras que en los países del sur, la agricultura es el principal consumidor (80 % en 2017). En general, las aguas superficiales contribuyen al 76 % del balance anual de agua, y las aguas subterráneas a alrededor del 24 % (Figura 3a; European Environment Agency, 2021, 2022).

El uso anual de agua en los Estados miembros de la UE disminuyó un 15 % entre 2000 y 2019 (European Environment Agency, 2022). La eficiencia en el uso del agua aumentó en los sectores dependientes del agua (p.e., la agricultura, la producción de energía, el suministro de agua para uso doméstico y el turismo) y la disponibilidad creció un 20 % del valor añadido neto (European Environment Agency, 2021). Esta disminución del consumo de agua también está relacionada con la sustitución de las centrales térmicas, que requieren agua para su refrigeración, por fuentes de energía renovables, como parte del nuevo Pacto Verde Europeo (European Commission, 2019b).

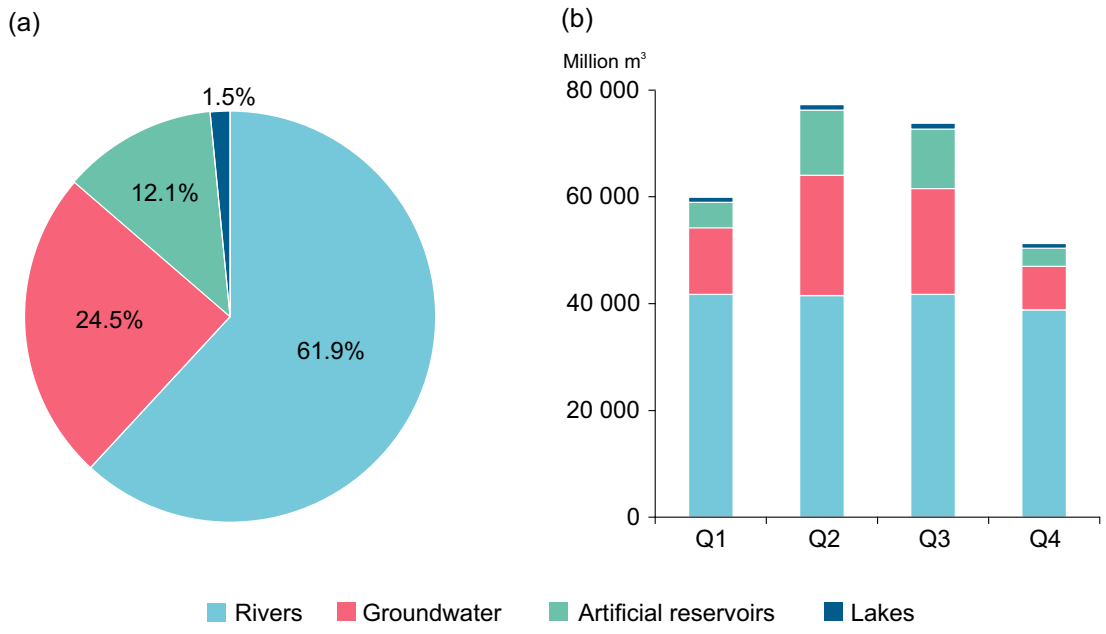


Figura 3 (a) Extracción anual y (b) estacional de agua dulce por fuentes en Europa (EEE-38 y Reino Unido). Q1: Enero, febrero y marzo, Q2: abril, mayo y junio, Q3: julio, agosto y septiembre, Q4: Octubre, noviembre y diciembre (European Environment Agency, 2021).

Sin embargo, la contribución relativa de las aguas subterráneas creció durante el mismo periodo. Este aumento (del 19 % en 2000 al 23 % en 2019) estuvo motivado por el creciente estrés hídrico al que se ven sometidas los cuerpos de agua superficiales, exacerbado por el cambio climático, especialmente en el sur de Europa durante la primavera y el verano (Figura 3b; European Environment Agency, 2021).

Las presas y su impacto ecológico

Con el fin de satisfacer las numerosas y diversas demandas de agua de nuestras sociedades actuales, se ha optado desde hace varias décadas por la construcción de grandes presas y embalses, azudes de derivación y otras infraestructuras de explotación del agua (Tharme, 2003). Su función principal es almacenar el agua durante periodos de caudal alto y liberarla gradualmente durante los periodos de estiaje, cuando es más necesaria (Baxter, 1977). En el caso de las presas hidroeléctricas, como la demanda de energía varía cada día y a lo largo del día, el agua puede retenerse durante la noche o en las épocas de mayor caudal y liberarse gradual o bruscamente, en función de las necesidades de electricidad (p.e., demandas base o punta; Egré and Milewski, 2002).

El número de grandes presas en todo el mundo ($\geq$15 m de altura, que embalsan más de 3 hm^3) pasó de 5.000 en 1950 a más de 58.000 en 2020 (ICOLD, 2021) y, en la actualidad, solo el 37 % de los grandes ríos de todo el mundo siguen conservando intacto su cauce (Grill et al., 2019). Este rápido crecimiento en la construcción de presas puede atribuirse no solo a la elevada demanda de agua y energía, sino también a la necesidad de reducir las emisiones de gases de efecto invernadero y a la adopción de fuentes de energía renovables como parte de las iniciativas de desarrollo sostenible (Zarfl et al., 2014).

Estas estructuras hidráulicas producen importantes impactos en los ríos y en las cuencas que los albergan. Entre las numerosas consecuencias de su construcción, que varían según el tamaño, la ubicación y el régimen de funcionamiento de las presas (Poff and Hart, 2002), las presas son responsables de alterar la dinámica de los caudales líquidos (Poff et al., 2007) y sólidos aguas abajo (Magilligan and Nislow, 2005), los ciclos biogeoquímicos (Maavara et al., 2020), la temperatura del agua (Olden and Naiman, 2010), e interrumpir la conectividad lateral y longitudinal de los cursos fluviales (Barbarossa et al., 2020; Grill et al., 2015).

Finalidad de las presas

Las presas para abastecimiento liberan agua en función de la demanda urbana, agrícola e industrial. Dependiendo de su tamaño, estas presas pueden modificar por completo los patrones estacionales del régimen de caudales, ya que, por norma general, acumulan los caudales durante la estación húmeda para liberarlos durante la estación seca, principalmente para sostener la agricultura de regadío (Richter and Thomas, 2007; Schmutz and Moog, 2018). En la mayoría de los casos, el agua embalsada se desvía directamente del embalse, lo que reduce significativamente la

cantidad de agua que circula por el cauce aguas debajo de la presa (Richter and Thomas, 2007).

Las grandes presas hidroeléctricas suelen tener una capacidad de almacenamiento considerable para acoplar el turbinado a las demandas energéticas, provocando cambios bruscos en los patrones hidrológicos aguas abajo (Poff and Hart, 2002). La generación intermitente de energía da lugar a frecuentes pulsos de caudal, los denomina nados "hidropeaks" (Greimel et al., 2018). Estas variaciones artificiales de caudal provocan fluctuaciones extremas y repentinas en el cauce del río, que se traducen en importantes reajustes geomorfológicos del mismo, cambios en los patrones de profundidad y velocidad del agua, o modificaciones significativas de la composición del material en suspensión, entre otros numerosos efectos (Moog, 1993; Schülting et al., 2019). En cambio, las presas hidroeléctricas denominadas "de pasada" tienen poca o ninguna capacidad de almacenamiento (Richter and Thomas, 2007). La ausencia de embalse reduce considerablemente los impactos ecológicos y sociales; sin embargo, su producción de energía puede variar significativamente durante el año, ya que depende del caudal natural del río (Egré and Milewski, 2002).

Las presas de control de inundaciones capturan una parte de los caudales de avenida para liberarlos posteriormente a un ritmo de descarga inferior (Richter and Thomas, 2007). Su principal objetivo es retrasar el proceso en el que las precipitaciones se convierten en escorrentía y llegan al río, contribuyendo también a desincronizar la llegada de los caudales máximos de los afluentes al eje principal (Green et al., 2000).

Por último, los embalses pueden construirse para crear una nueva masa de agua estancada con fines económicos o sociales, como la disipación del calor en una central termoeléctrica, la pesca, el turismo, la recreación y los valores religiosos (Baxter, 1977; Richter and Thomas, 2007).

Principales impactos causados por las presas

El proceso de represamiento de los ríos y embalsamiento de las aguas crea ecosistemas diferentes de los que había en los arroyos y lagos que los acogen (Baxter, 1977). El régimen de caudales y su dinamismo son los factores fundamentales que definen la estructura física y los procesos ecológicos en los ríos (Poff et al., 1997). Por tanto, la alteración del régimen de caudales provocada por las presas causan cambios inmediatos en otros componentes del ecosistema, como los flujos de energía y materia, la conectividad y la distribución de la biota.

Alteración del régimen de caudales

El paradigma del régimen natural de caudales (sensu Poff et al., 1997) establece que el régimen de caudal, que puede describirse mediante cinco atributos ecológicos (magnitud, frecuencia, duración, temporalidad y tasa de cambio), sustenta la biodiversidad y la integridad de los ecosistemas acuáticos (Richter et al., 1996, 1997). Sin embargo, las presas tienden a homogeneizar la variabilidad de los caudales principalmente mediante cambios en la magnitud, frecuencia y periodicidad de los caudales altos y bajos (Magilligan and Nislow, 2005; Poff et al., 2007). Por ejemplo, los caudales altos que se dan en régimen natural, además de transportan organismo y permitir que especies colonicen zonas aguas abajo (Ward and Stanford, 1995), contribuyen a exportar sedimentos y material orgánico (Schmutz and Moog, 2018). Aunque esto es sólo un ejemplo, la alteración de los atributos del régimen de caudales puede dar lugar a una amplia gama de respuestas en diferentes elementos ecológicas y geomorfológicas (Lloyd et al., 2004; Poff and Zimmerman, 2010).

El vínculo entre el caudal y la biota puede resumirse con los principios clave caudal-biota propuestos por Bunn and Arthington (2002). La morfología fluvial viene determinada en gran medida por la interacción entre el régimen de caudales y la geomorfología local; simultáneamente, la forma y el tamaño del río fijan la distribución y diversidad de la biota (Principio 1), y la estacionalidad del caudal determina los ciclos vitales de las especies que lo habitan (Principio 2). Los episodios de avenida pueden conectar hábitats, ampliar la red fluvial y permitir a las especies acceder a nuevas zonas (Principio 3). Así pues, dado que los tres primeros principios establecen las condiciones ideales para las especies nativas, la modificación de las condiciones de caudal puede provocar la pérdida de biodiversidad y facilitar las invasiones de especies exóticas (Principio 4).

Flujos de sedimentos y nutrientes

La retención de sedimentos es otro impacto muy significativos de presas y embalses (Schmutz and Moog, 2018), que se está viendo agravado por la construcción acelerada de presas y los cambios en el uso del suelo en todo el mundo. Recientemente, Dethier et al. (2022) informaron de una reconfiguración de los patrones globales en los flujos de sedimentos hacia los océanos, que indican que en el hemisferio norte la retención de sedimentos producida por las presas ha reducido en casi la mitad el flujo de sedimentos que llega al mar.

A medida que las presas interrumpen la continuidad del transporte de sedimentos a lo largo del sistema fluvial, los sedimentos se van acumulando en el embalse, lo que provoca una pérdida de su capacidad de almacenamiento, obstruye las estructuras de

salida y disminuye la eficiencia de las turbinas y otros equipos hidráulicos (Schleiss et al., 2016). Al mismo tiempo, los tramos aguas abajo se ven privados de los sedimentos, que son esenciales para sustentar los ecosistemas, aumenta la rugosidad del lecho y se modifican hábitats críticos, p.e., las camas de freza del salmón y la trucha y otras especies piscícolas (Kondolf, 1997; Kondolf et al., 2014).

Del mismo modo, el flujo y el transporte de nutrientes (carbono, fósforo, nitrógeno, silicio) se ven bloqueados por las presas, lo que genera una modificación de sus ratios a consecuencia de la deposición en los sedimentos o la liberación de gases a la atmósfera (Maavara et al., 2020). Esto tiene importantes consecuencias sobre el funcionamiento y la biodiversidad de los ecosistemas, alterando el metabolismo fluvial (González et al., 2013; von Schiller et al., 2016), la estructura de las redes tróficas a todos los niveles (Glibert, 2012), la reducción de la densidad de macroinvertebrados (Kennedy and Turner, 2011) y las poblaciones de salmón (Moore et al., 2011).

Temperatura del agua

La temperatura del agua es una variable física clave en los ecosistemas fluviales, que influye en el metabolismo fluvial, la distribución de la biodiversidad, el ciclo biológico y las tasas de crecimiento de la biota (Buisson et al., 2008), p.e., el crecimiento de algunos macroinvertebrados y el desarrollo de sus huevos (Elliott, 1978; Vannote and Sweeney, 1980) o el desove y desarrollo de los salmónidos (Flodmark et al., 2004; Isaak et al., 2010).

Las presas y los embalses alteran el régimen térmico de los ríos a través de numerosas vías (Niemeyer et al., 2018). Los grandes embalses (con presas $\geq$15 m de altura) tienden a sufrir una estratificación térmica debido a que la densidad del agua varía con la temperatura. En verano, una capa superficial relativamente cálida (epilimnion) se "separa" del agua más fría y densa (hipolimnion; Petts, 1986). Así, en los embalses con descarga de fondo, el agua desembalsada produce una reducción de la temperatura del agua aguas abajo (Hester and Doyle, 2011). Por el contrario, en presas pequeñas (p.e., presas hidroeléctricas de pasada), se espera que la temperatura aumente aguas abajo debido a la menor velocidad, el aumento de la superficie del embalse y el efecto de la radiación solar (Chandesris et al., 2019).

Los cambios en el régimen de temperaturas inducidos por presas tienen una gran influencia en la biota. Estudios previos han demostrado que la constancia de la temperatura del agua aguas abajo de las presas es un factor crítico para la persistencia de los macroinvertebrados y, por tanto, la riqueza taxonómica de esos tramos (Rader et al., 2008). El aumento de las temperaturas como consecuencia de

la presencia de una presa también se ha relacionado con una disminución de especies de peces de agua fría (Hayes et al., 2006; Lessard and Hayes, 2003).

Aunque los impactos de los cambios térmicos rara vez se reconocen en las estrategias de mitigación de los impactos de las presas (Olden and Naiman, 2010), el diseño e instalación de múltiples puntos de desembalse que abarquen toda la profundidad del embalse pueden desembalsar agua desde diferentes profundidades en determinados periodos del año, lo que permite imitar la variación natural de la temperatura a lo largo del año en los tramos afectados (Rader et al., 2008).

Conectividad fluvial

Las presas y azudes funcionan como barreras artificiales que impiden o dificultan el movimiento longitudinal a lo largo de la red fluvial, que se ve amplificado por la propia configuración dendrítica de la misma (Crook et al., 2015). Las presas, por tanto, perturban los patrones naturales de dispersión de los organismos, alterando la estructura, composición y dinámica de muchas poblaciones locales (Rolls et al., 2013). Esto afecta directamente a la ictiofauna y especialmente a las especies que realizan movimientos migratorios a lo largo de la red fluvial y son totalmente dependientes de las conexiones hidrológicas del sistema (Agostinho et al., 2008; Landeiro et al., 2011). Estudios anteriores han puesto de manifiesto una acumulación de juveniles en tramos situados directamente debajo de las presas que bloquean sus rutas migratorias, así como una reducción de la riqueza taxonómica de peces (Gehrke et al., 2002). González-Ferreras et al. (2022) también encontraron que la presencia de barreras causaba una fuerte disminución de la diversidad genética y del tamaño poblacional de la trucha común en tramos aislados por estas barreras artificiales. Los insectos voladores o las diatomeas, por el contrario, pueden verse menos afectados por la fragmentación del hábitat, ya que son capaces de llegar a otras ramas de la red fluvial o incluso a cuencas vecinas (Liu et al., 2013).

Algunos cambios estructurales u operativos, como la instalación de pasos para peces y la liberación periódica de caudales de avenida habituales, pueden reducir los problemas de conectividad (Bravo-Córdoba et al., 2022). Las escalas de peces consisten en una vía abierta por donde se desvía parte del caudal para que los peces, tales como los salmónidos y otras especies anádromas, alcancen los hábitats de desove y cría aguas arriba. En el caso de las grandes presas, los peces también pueden ser trasladados aguas arriba mediante ascensores para peces (Schilt, 2007). Sin embargo, estas estructuras pueden no tener éxito debido a diseños fallidos, que impiden a los peces localizar las entradas o presentan tamaño de paso, pendiente o velocidades inadecuadas (Jonsson and Jonsson, 2011). Por ejemplo, solo el 47 % de los salmones

consiguen remontar río arriba a través de un ascensor para peces en el río Garona (Francia), ya que el efecto de atracción hacia su entrada era insuficientemente (Croze et al., 2008), y la migración del salmón se retrasó considerablemente debido al bajo caudal en un paso para peces en Noruega (Thorstad et al., 2003).

La eliminación de presas está ganando relevancia en la restauración fluvial (Magilligan et al., 2016; O'Connor et al., 2015), aunque estas acciones suelen estar sujetas a intereses socioeconómicos, p.e., una producción de energía ineficiente y no rentable, estructuras inseguras o presas abandonadas (Bednarek, 2001). La eliminación de barreras proporciona importantes ventajas medioambientales al restablecer los flujos de sedimentos, nutrientes y organismos en ambas direcciones (Magilligan et al., 2016). Sin embargo, también puede tener implicaciones negativas, como el paso de especies introducidas (p.e., el control de las poblaciones de lamprea marina en los Grandes Lagos de Norteamérica; Miehls et al., 2020) o la liberación de sedimentos contaminados (Bednarek, 2001).

Mitigación del impacto de las presas: caudales ecológicos

Teniendo en cuenta los impactos de las presas y embalses en los ecosistemas de agua dulce y los retos relacionados con el nexo agua-energía-alimentación a los que se enfrentan las sociedades, han surgido diferentes estrategias para reducir los múltiples efectos que estas infraestructuras hídricas producen en la salud de los ecosistemas fluviales.

Las primeras iniciativas hacia la protección de las masas de agua surgieron a finales de la década de los 40s en Estados Unidos en los arroyos de montaña para proteger la pesca de especies de agua fría (Poff et al., 2017). Otros esfuerzos llevados a cabo en esa época en Estados Unidos y Europa occidental se limitaron a establecer caudales mínimos para mitigar el deterioro de la calidad del agua (Poff et al., 1997). En la década de los 70s, se produjeron avances significativos en la conservación de los ríos, mediada por el desarrollo de una nueva legislación en Estados Unidos (Ley de Agua Limpia; 92nd U.S. Congress, 1972) ante el auge de la construcción de presas. A partir de la década de 90s, el desarrollo de nuevos conceptos en conservación y protección de los ecosistemas acuáticos y su puesta en práctica sufrieron una fuerte expansión a escala mundial (Poff et al., 2017).

La Declaración de Brisbane de 2007, y su posterior revisión en 2018a, representa una iniciativa fundamental para promover la implantación de caudales ecológicos a escala global. Según esta Declaración, el término caudales ecológicos (o "e-flows") se

define como "la cantidad, el momento y la calidad de los caudales y niveles de agua dulce necesarios para mantener los ecosistemas acuáticos que, a su vez, sustentan las culturas humanas, las economías, los medios de vida sostenibles y el bienestar". En esta Declaración, científicos, entidades conservacionistas y gestores del agua establecen recomendaciones para apoyar la implementación de caudales ecológicos, que representan un elemento crítico para alcanzar los ODS relacionados con el agua (Tickner et al., 2020a). En este contexto, el reconocimiento que ciertos requisitos relacionados con el caudal son esenciales para sostener la estructura y la función de todo el ecosistema fluvial y los medios de vida locales condujo al desarrollo de métodos holísticos para la definición de regímenes de caudal ecológico (Poff et al., 2017; Zeiringer et al., 2018), p.e., los Límites Ecológicos de la Alteración Hidrológica (ELOHA de sus siglas en inglés, Figura 4; Poff et al., 2010). Este marco de actuación, ampliamente aceptado por científicos de todo el mundo, se basa en el seguimiento hidrológico y biológico, la agrupación y clasificación de los ríos, la generación de relaciones entre la alteración del caudal y las respuestas ecológicas transferibles entre tramos fluviales, la definición de estrategias de gestión adaptativa, considerando las necesidades y objetivos de la sociedad.

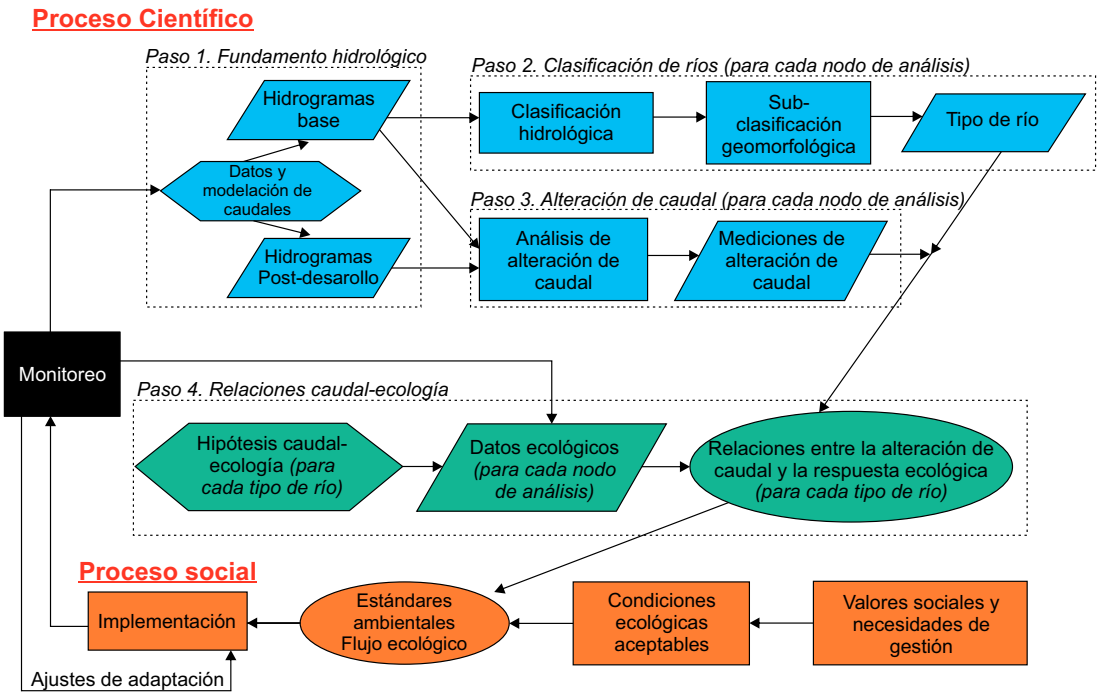


Figura 4 Resumen del ELOHA, un marco holístico en el que se tienen en cuenta aspectos hidrológicos, ecológicos y sociales para evaluar los efectos de la alteración hidrológica (Poff et al., 2010).

Retos en la evaluación e implementación de caudales ecológicos

Aunque los requisitos medioambientales del agua se están incorporando a las políticas de alto nivel relacionadas con gestión de las cuencas y la protección de los ecosistemas acuáticos, p.e. la DMA (European Commission, 2000), la Ley Nacional del Agua de Australia (Australian Government, 2007), y la legislación sobre evaluación del impacto ambiental en China (Chen and Wu, 2019), todavía existen impedimentos significativos que les impiden lograr sus objetivos (Arthington et al., 2018b). Entre ellos, un nivel inadecuado de apoyo político y compromiso de las partes interesadas, recursos insuficientes (p.e., financiación para los estudios técnicos de cálculo de caudales ecológicos, así como su monitoreo y gestión), falta de datos y conocimiento (p.e., se necesitan datos de caudal, geomorfológicos, ecológicos y socioeconómicos para la evaluación y la implementación), barreras institucionales y conflictos de intereses (Harwood et al., 2017; Le Quesne et al., 2010).

Aunque es bien sabido que los diferentes atributos del régimen de caudales y su dinamismo son esenciales para los ecosistemas acuáticos, la mayoría de los aproximaciones relacionadas con caudales ecológicos se centran en el mantenimiento de los caudales mínimos (Tharme, 2003). Los caudales mínimos suelen ser un valor de definido por las autoridad de cuenca o la legislación nacional y, en la mayoría de las ocasiones, distan mucho de ser adecuados para la biota acuática, ya que sólo cubren una de las diversas demandas del ecosistema (Jager and Smith, 2008). En el caso de los ríos de llanura de inundación, p.e., las recomendaciones de caudal ecológico que garanticen la conectividad entre la llanura de inundación y el cauce del río son esenciales, por la consideración de aquellos componentes del caudal (p.e., la magnitud de los caudales mínimos o la frecuencia y el temporalidad de los pulsos de caudal alto) que mantienen la conectividad, la temperatura del agua, el flujo de sedimentos y nutrientes son esenciales a la hora de definir los caudales ecológicos (Hayes et al., 2018).

Además, desde una perspectiva más amplia, las aproximaciones más habituales para el cálculo de caudales ecológicos priorizan el régimen de caudales líquidos (es decir, la alteración hidrológica como principal impacto relacionado con las presas) a expensas de otros elementos esenciales (p.e., temperatura, sedimentos y conectividad; Olden and Naiman 2010; Wohl et al. 2015). Por tanto, en muchos casos, el restablecimiento del caudal no es suficiente para restablecer la integridad del ecosistema aguas abajo de las presas (Poff, 2018). En un ejemplo de aplicación de ELOHA, McManamay et al. (2013) aconsejaron la consideración de elementos adicionales además del régimen de caudal, incluyendo la temperatura, la pendiente y los sedimentos, para mantener relaciones cruciales entre el caudal y el funcionamiento

del ecosistema, ya que pueden ser críticas para el éxito de la recolonización de la ictiofauna de esos ríos.

La intermitencia de caudal tampoco suele tenerse en cuenta a la hora de definir caudales ecológicos, aunque los ríos temporales y efímeros son comunes en las redes fluviales mediterráneas (Stubbington et al., 2018). La complejidad de las relaciones entre eventos de intermitencia con las comunidades biológicas, su variabilidad temporal y espacial, y la escasez de datos, son las principales dificultades a la hora de abordar la gestión de los ríos temporales (Acuña et al., 2020). Las estaciones de aforo rara vez se localizan en estas masas de agua (<5 % en Portugal y <1 % en España; Stubbington et al., 2018) y el gran dinamismo espacio-temporal limita la aplicación de los enfoques de monitorización biológica más habituales, p.e., relacionado con la ausencia de valores de referencia o la clasificación errónea del estrés antropogénico (Cid et al., 2020).

La dispersión de organismos también es un proceso relevante en este contexto, dada su influencia en la estructura de la comunidad, especialmente en ecosistemas altamente dinámicos (Cid et al., 2020; Poff, 2018). Los movimientos activos para localizar hábitats adecuados y colonizar nuevos tramos o la dispersión de propágulos crean un flujo de individuos, por lo que las comunidades locales se vinculan entre sí a través de la dispersión, formando una metapoblación (Brown and Swan, 2010; Fagan, 2002). Sin embargo, esta cuestión no se suele tener en cuenta en las estrategias de conservación (Cid et al., 2020; Poff, 2018).

En resumen, la ciencia de los caudales ecológicos debe incorporar una serie de elementos nuevos en el análisis y las recomendaciones ambientales (relacionados directa o indirectamente con la hidrología). Esto toma más relevancia cada día, a medida que avanzamos hacia un periodo en que factores dinámicos (p.e., el cambio climático y la demanda de agua) tienen mayor influencia y general mayores impactos en las condiciones hidrológicas y ambientales futuras y, consecuentemente, en los ecosistemas acuáticos (Poff, 2018).

Indicadores biológicos para evaluar el impacto de las presas

El uso de la biota para evaluar y monitorizar los cambios en las aguas corrientes se originó en la segunda mitad del siglo XIX, principalmente para controlar las enfermedades infecciosas (Bonada et al., 2006). Hacia 1900, las comunidades acuáticas (incluidos macrófitos, macroinvertebrados y peces) se emplearon por primera vez como indicadores de la contaminación de las aguas dulces en Alemania, motivado

por la ineficacia de los criterios químicos para detectar los efectos de factores de estrés o fuentes de contaminación sobre la calidad del agua, en las que los contaminantes vertidos no estaban presentes (Bonada et al., 2006; Yoder and Rankin, 1998). Hoy en día, el conocimiento de la respuesta de varios grupos bióticos (p.e., diatomeas, fitoplancton, macrófitos, macroinvertebrados y peces) a cambios en el hábitat (Hauer and Resh, 2017; Marcel et al., 2017), a la configuración del ecosistema (p.e., evolución longitudinal del río; Vannote et al., 1980), y a diferentes factores de estrés antropogénicos (p.e., contaminación, presencia de presas y embalses, especies invasoras; Dudgeon, 2019) ha favorecido el establecimiento del monitoreo biológico (o biomonitoreo) como un componente esencial en la gestión y conservación de las aguas dulces y los servicios ecosistémicos que proporcionan (Friberg et al., 2011).

Los impactos de las presas, la eficacia de los caudales ecológicos y otras estrategias de conservación pueden evaluarse eficazmente mediante las comunidades biológicas, debido a su gran diversidad y al amplio espectro de respuestas a lo largo de sus ciclos vitales (Bonada et al., 2006). Por ejemplo, se han utilizado peces para evaluar la eficacia de los sistemas de derivación para acceder al hábitat de desove en ríos afectados por presas hidroeléctricas (Nagel et al., 2021), comunidades de macroinvertebrados parea evaluar los efectos del lavado de sedimentos por efecto de presas (Folegot et al., 2021), y comunidades de macrófitos y diatomeas para evaluar los efectos de la alteración hidrológica (Goldenberg-Vilar et al., 2021).

La mayoría de los programas de monitorización biológica tienen múltiples objetivos y trabajan a diferentes escalas espaciales (locales y regionales) y temporales (Resh, 2008). Por lo tanto, se recomienda el uso de múltiples comunidades y métricas para evaluar el efecto de diferentes presiones (Resh, 2008; Soininen and Könönen, 2004). Por ejemplo, la ubicuidad de ciertos organismos, como las diatomeas, los insectos y algunas especies de peces, los convierte en excelentes indicadores de las condiciones del hábitat local, mientras que los peces de mayor tamaño y los macroinvertebrados no insectos suelen ser mejores indicadores de la conectividad debido a su movilidad restringida a través de la red fluvial (Figura 5; Lammert and Allan, 1999; Mangadze et al., 2016).

Dado que las normativas ambientales aplican, a menudo, a grandes extensiones geográficas (p.e., la DMA en el ámbito europeo), las herramientas de biomonitorización requieren una redefinición o re-escalado que asegure su correcta aplicación en múltiples biorregiones (Statzner et al., 2001). En este contexto, los rasgos de los organismos representan una herramienta con el potencial necesario para unificar las las valoraciones basadas en taxonomía y desarrollar reglas generales y comparables para explicar y predecir los procesos ecológicos (Funk et al., 2017). Los rasgos se definen como características que reflejan la adaptación de las especies al medio

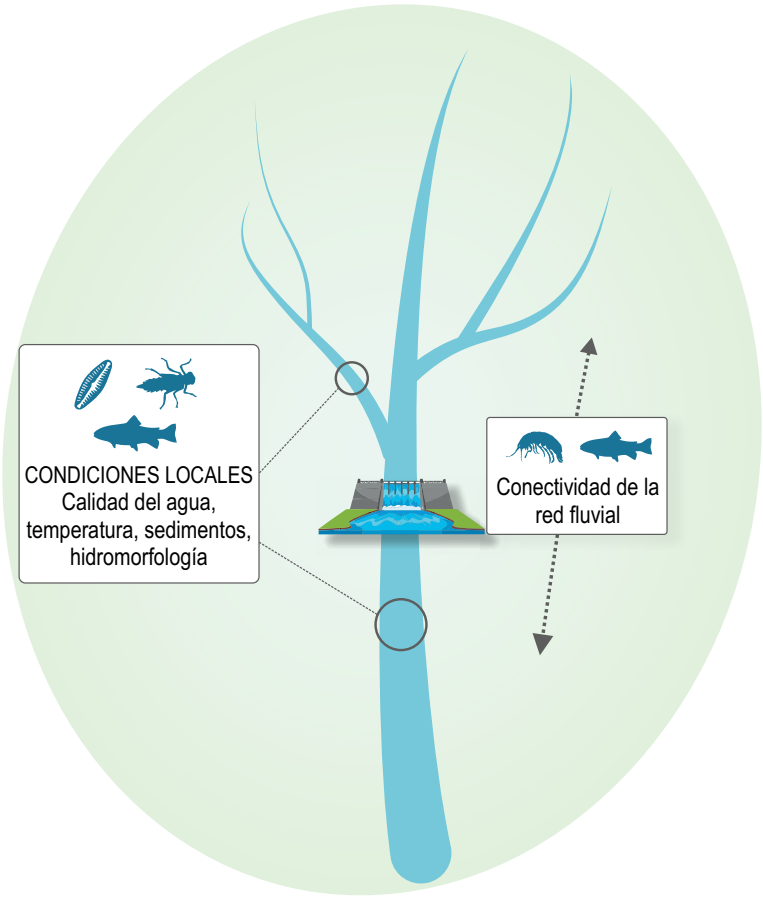


Figura 5 Bioindicadores utilizados para evaluar los impactos producidos por presas a escala local (p.e., cambios en la calidad del agua, los sedimentos y la hidromorfología utilizando diatomeas, macroinvertebrados y peces) y a escalas espaciales más amplias (grado de conectividad utilizando macroinvertebrados no insectos y peces).

ambiente, y pueden dividirse en rasgos biológicos (p.e., tamaño, hábitos alimentarios y estrategias reproductivas) y ecológicos (p.e., preferencias de temperatura, tolerancia a la contaminación; Menezes et al., 2010). Desde los primeros trabajos en ecología vegetal que abordaban cómo las perturbaciones determinaban los rasgos de la vegetación (Grime, 1974), la investigación basada en rasgos ha ido creciendo en la literatura científica (Menezes et al., 2010). En las últimas décadas, los rasgos han tomado mayor protagonismo para conectar los mecanismos basados en el nicho con los patrones de las comunidades, transformando la modelización de la distribución de las especies y la dinámica de las poblaciones (Cadotte et al., 2015; Dawson et al., 2021).

Diatomeas

Las diatomeas (algas unicelulares con paredes celulares silíceas) se han utilizado ampliamente en la monitorización de ecosistemas fluviales (Passy, 2007; Potapova

and Charles, 2002; Reid et al., 1995). Su gran biodiversidad y ubiquidad, su papel fundamental en las redes tróficas y ciclos biogeoquímicos, y sus respuestas directas a los cambios químicos y físicos convierten a las comunidades de diatomeas en valiosos indicadores de las condiciones ambientales (Stevenson and Sabater, 2010).

Existe una amplia bibliografía sobre las diatomeas en hábitats marinos, costeros y lénticos, lo que destaca la relevancia de las diatomeas como bioindicadores (p.e., Birks et al., 1990; Krajenbrink et al., 2019; Pandey et al., 2017; Rimet and Bouchez, 2011). Además, en la última década, las comunidades de diatomeas también se han utilizado para investigar el efecto de las presas y otros cambios hidromorfológicos en los ríos (p.e., Gillett et al., 2016; Goldenberg-Vilar et al., 2021; Wang et al., 2022; Wu et al., 2019b).

Se han desarrollado varios índices bióticos basados en las comunidades de diatomeas para evaluar la calidad y la contaminación del agua, destacando el Índice Biológico de Diatomeas (BDI; AFNOR, 2000) o el Índice de Sensibilidad a la Contaminación (IPS; CEMAGREF, 1982). Estos índices, que también se utilizan en la evaluación de las masas de agua según la DMA, se basan en la sensibilidad a la contaminación de determinadas especies de diatomeas. Sin embargo, la precisión de estos índices bióticos se puede ver mermada por la dificultad en el procesado de las muestras, asociado principalmente a la identificación de especies raras, a la taxonomía inestable y rápidamente cambiante de este grupo, y a la variabilidad de sus preferencias ecológicas según la región biogeográficas (Berthon et al., 2011; Hlúbiková et al., 2014).

Por lo tanto, el enfoque de evaluación basado en clasificaciones de rasgos funcionales desarrollado por Passy (2007), se utiliza frecuentemente como alternativa a las métricas tradicionales basadas en la taxonomía de este grupo. Estas clasificaciones combinan información sobre la tolerancia de las diatomeas a la limitación de nutrientes, la capacidad de asimilar recursos y la resistencia a las perturbaciones (Soininen et al., 2016). La clase funcional denominada *perfil bajo* se compone de diatomeas de pequeño tamaño asociadas a ambientes con concentraciones bajas de nutrientes y que habitan dentro de la capa de biofilm, lo que las confiere mayor resistencia a las perturbaciones físicas. Por el contrario, debido a su forma alargada y porte alto, las diatomeas de *perfil alto* pueden acceder fácilmente a los nutrientes y la luz, pero son más susceptibles a las perturbaciones (p.e., corriente o herbívoros). La clase *móvil* está formada principalmente por especies tolerantes a la eutrofización y a la contaminación, tiene la capacidad de buscar activamente hábitat más adecuado y, por lo tanto, no se encuentra coartada por de limitaciones de recursos o por el régimen de perturbaciones (Passy, 2007). Por último, Rimet and Bouchez (2012)

propusieron la clase *planctónica*, que consiste en diatomeas adaptadas a ambientes lénticos con características morfológicas para evitar la sedimentación.

Macroinvertebrados

Por lo general, los ríos y arroyos presentan una importante diversidad dentro del grupo de los macroinvertebrados, que se compone principalmente de artrópodos (insectos, ácaros, crustáceos), moluscos (caracoles, mejillones, almejas), anélidos (gusanos segmentados y sanguijuelas), nematodos (ascárides), entre otros (Carter et al., 2017; Hauer and Resh, 2017). Las especies de macroinvertebrados son principalmente bentónicas (es decir, asociadas a superficies del fondo del cauce, como la roca madre, los cantos, los sedimentos finaos u otras superficies estables; Hauer and Resh, 2017).

Los macroinvertebrados son los organismos más utilizados en el seguimiento y monitorización de ecosistemas lóticos (Bonada et al., 2006) y su abundancia, riqueza taxonómica y la distribución de especies en la comunidades ha sido objeto de investigación en numerosos estudios sobre calidad del agua (Álvarez-Cabria et al., 2010). Por lo tanto, su ubicuidad, su taxonomía bien conocida, el gran número de especies sensibles a las perturbaciones y la facilidad de muestreo hace que sean perfectos candidatos para ser utilizados como bioindicadores (Carter et al., 2017).

Los índices bióticos de macroinvertebrados también se utilizan en gran medida para medir la contaminación orgánica. Como ejemplo, el Biological Monitoring Working Party (BMWP; Armitage et al., 1983) se desarrolló en el Reino Unido para evaluar la contaminación fluvial a escala nacional, y se basa en la tolerancia de todas las familias de macroinvertebrados bentónicos de una muestra. El BMWP se ha utilizado y se utiliza ampliamente en Europa, con adaptaciones nacionales o regionales que consideran nuevas familias y las otorgan índices de sensibilidad, p.e. en los países ibéricos (IBMWP; Alba-Tercedor et al., 2002). De forma similar, el índice ASPT (Average Score Per Taxon) calcula la media de las puntuaciones de tolerancia de todas las familias de macroinvertebrados y no depende de la riqueza de familias como el BMWP.

Los enfoques taxonómicos tradicionales incluyen índices de diversidad para evaluar la composición y estructura de las comunidades de macroinvertebrados. El índice de Shannon-Wiener tiene en cuenta la riqueza de especies y la abundancia relativa de las mismas, que da información sobre la dominancia y la estructura de la comunidad (valores más altos indican mayor homogeneidad en la abundancia relativa; Feld et al., 2014). La diversidad de Simpson también tiene en cuenta la riqueza de especies y sus abundancias relativas, representando su valor la probabilidad de que dos individuos

de una muestra no pertenezcan a la misma especie (valores más altos indican una comunidad más diversa; Feld et al., 2014).

A diferencia de la mayoría de los índices bióticos que analizan la calidad y la contaminación del agua, el Índice de Invertebrado Lóticos para la Evaluación de Caudales (LIFE de sus siglas en inglés; Extence et al., 1999) relaciona las comunidades de macroinvertebrados fluviales con los regímenes de caudal predominantes. Esta métrica se aplicó sobre todo en el Reino Unido y está pensada para evaluar los impactos de los regímenes variables de caudal fluvial en las comunidades de macroinvertebrados (Extence et al., 1999; Laini et al., 2018).

Peces

Más de 10.000 especies de peces viven en aguas dulces, lo que representa, aproximadamente, el 40 % de todas las especies de peces (Lundberg et al., 2000). Las comunidades de peces son muy dinámicas y su distribución, composición y estructura varían ampliamente en el tiempo y espacio (Matthews, 1998).

A la hora de evaluar los cambios ambientales, las comunidades de peces ofrecen varias ventajas en comparación con otros grupos bióticos. Son vulnerables a una serie de factores de estrés relacionados con diversos componentes del hábitat que afectan al desove, la alimentación y la supervivencia, como la composición del sedimento, la presencia de vegetación o la temperatura del agua. Además, debido a su longevidad, es posible observar los efectos de las perturbaciones a lo largo de las distintas etapas de su vida, lo que proporciona registros a largo plazo del estrés ambiental (Fausch et al., 1990). Los movimientos migratorios de algunas especies a lo largo de la red fluvial pueden ser indicadores del grado de conectividad y fragmentación del hábitat, p.e., los salmónidos anádromos o las anguilas catádromas realizan largas migraciones, mientras que otras especies realizan movimientos cortos para acceder a los hábitats de desove o refugio (Matthews, 1998). Además, la larga tradición en investigación de la ecología, fisiológica y ecotoxicológica de peces ha contribuido a avanzar en el conocimiento de un gran número de especies y de sus requerimientos ecológicos, lo que los convierte en buenos indicadores para el seguimiento ambiental (Chovanec et al., 2003; Schiemer, 2000).

De esta manera, se han elaborado diversos índices bióticos locales basados en peces para evaluar la integridad de los ecosistemas fluviales a escala europea. Sin embargo, el alto grado de especificidad de las comunidades a escala de cuenca, la falta de información sobre especies concretas, la marcada variabilidad intra e interanual de las comunidades de peces y la escasez de ríos y arroyos prístinos (es decir, de tramos fluviales en condiciones de referencia) dificultan el desarrollo de métricas válidas en

escalas espaciales grandes (Aparicio et al., 2011; Ferreira et al., 2007; Segurado et al., 2014). En este contexto, el Índice Europeo de Peces (EFI+ de sus siglas en inglés; EFI+ Consortium, 2009) fue el primer método de evaluación estandarizado basado en los peces para su aplicación en todos los ríos europeos (Segurado et al., 2014). Se trata de un índice multimétrico que cuantifica la desviación entre los conjuntos de peces en condiciones de referencia y un conjunto de peces determinado, basándose en grupos funcionales que describen las principales características ecológicas y biológicas de los peces (EFI+ Consortium, 2009).

La situación en los ríos españoles

España se sitúa en el suroeste de Europa, con una superficie de 505.990 km^2 (Figura 6; Tapiador, 2020). Limita al norte con Francia a lo largo de los Pirineos y el Golfo de Vizcaya, al noroeste con Portugal y el Océano Atlántico, y al sur y al este con el Mar Mediterráneo. En su extremo sur, un estrecho de 13 km la separa del continente africano (Tapiador, 2020).

La combinación de su situación geográfica, clima y relieve hace de España un país biogeográficamente complejo. En cuanto a sus recursos hídricos, la disponibilidad de agua en España (alrededor de 40.000 $hm^3.año^{-1}$) es relativamente alta; sin embargo, se caracteriza por una distribución espacio-temporal irregular asociado a la situación geográfica del país, el relieve y los fuertes contrastes climáticos (Costejà et al., 2002; Ministerio de Medio Ambiente, 2004).

La diversidad natural del país

La España continental se caracteriza por una compleja configuración orográfica (Figura 6). Presenta una extensa y alta meseta interior (alrededor de 600 m.s.n.m.) dividida por el Sistema Central en la submeseta norte (altitudes aproximadas de 800-850 m.s.n.m.) y la submeseta sur (500-700 m.s.n.m.). La Meseta está rodeada al norte por la Cordillera Cantábrica (unos 2.600 m.s.n.m.), al este por el Sistema Ibérico (2.300 m.s.n.m.) y al sur por Sierra Morena (1.300 m.s.n.m.). Fuera de esta meseta central, los Pirineos y el Sistema Bético, dos cadenas montañosas notables, alcanzan los 3.400 m.s.n.m. (Rivas-Martínez et al., 2004; Suárez-Seoane et al., 2002). La geología se caracteriza, en general, por la dominancia de rocas silíceas en el oeste y formaciones calcáreas en el este de la Península Ibérica (Sabater et al., 2022).

El Océano Atlántico y el Mar Mediterráneo ejercen una fuerte influencia sobre el clima del país (Figura 7a). El norte y el oeste están muy expuestos a los vientos

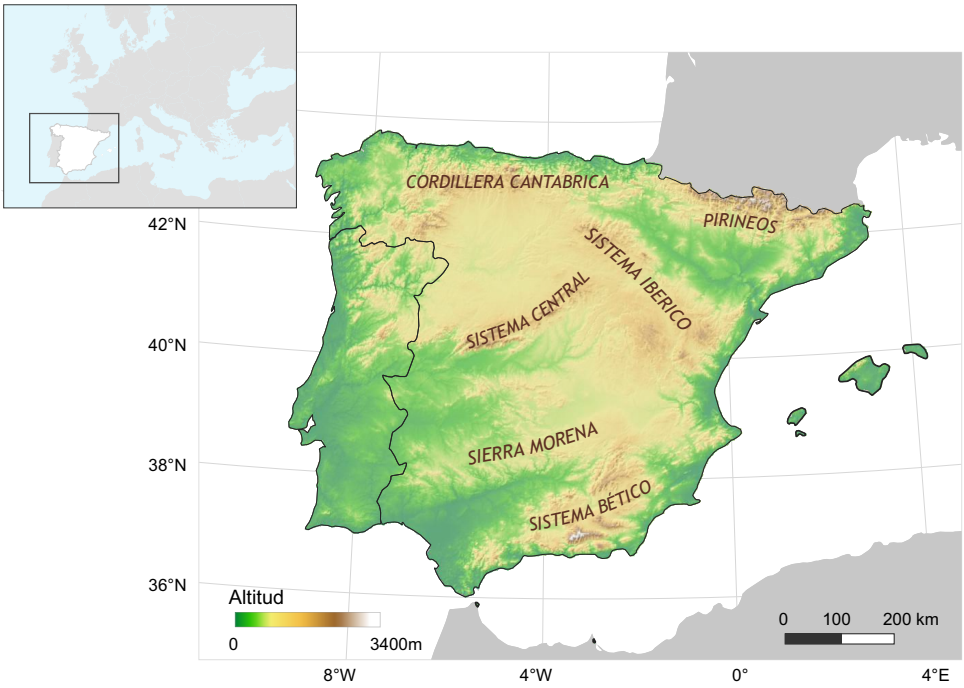


Figura 6 Localización de España en Europa, su orografía y los principales accidentes geográficos.

marítimos atlánticos y se caracterizan por veranos suaves e inviernos húmedos (p.e., Vigo y Santander en Figura 7). Las zonas montañosas presentan climas fríos y precipitaciones abundantes. La parte meridional es la región más cálida y seca, ya que recibe la influencia de las masas de aire cálido procedentes del mar Mediterráneo y del desierto del Sáhara (p.e., Cádiz y Murcia en la Figura 7; AEMET and IMP, 2011). Las precipitaciones (Figura 7b) presentan un marcado gradiente espacial, coincidiendo las zonas húmedas con las cabeceras de montaña (Cardoso Pereira et al., 2020) y se concentran en la estación fría en las regiones atlánticas y en primavera y otoño en las zonas mediterráneas (López-Moreno et al., 2008).

La vegetación también refleja la diversidad climática presente en el país. Las regiones fitoclimáticas determinadas por Allué (1990) describen la amplia gama de condiciones bioclimáticas presentes en el país. En las áreas de montaña (fitoclimas oroboreales), la vegetación se caracteriza por coníferas, p.e., el *Pinus uncinata* en los Pirineos, el *Pinus sylvestris* en las sierras del Sistema Central y Teruel, y pastizales alpinos y subalpinos (Bedia et al., 2014; Tapiador, 2020). Los bosques caducifolios están presentes en las regiones con influencia atlántica, con precipitaciones elevadas y temperaturas suaves (fitoclimas nemorales), p.e., el quejigo (*Quercus faginea*) en zonas húmedas y frías, el roble negro *(Quercus pyrenaica*) en zonas montañosas menos húmedas de todo el norte del país, y el haya (*Fagus sylvatica*) en zonas montañosas frescas y húmedas (Bedia et al., 2014; Tapiador, 2020). En las regiones semiáridas

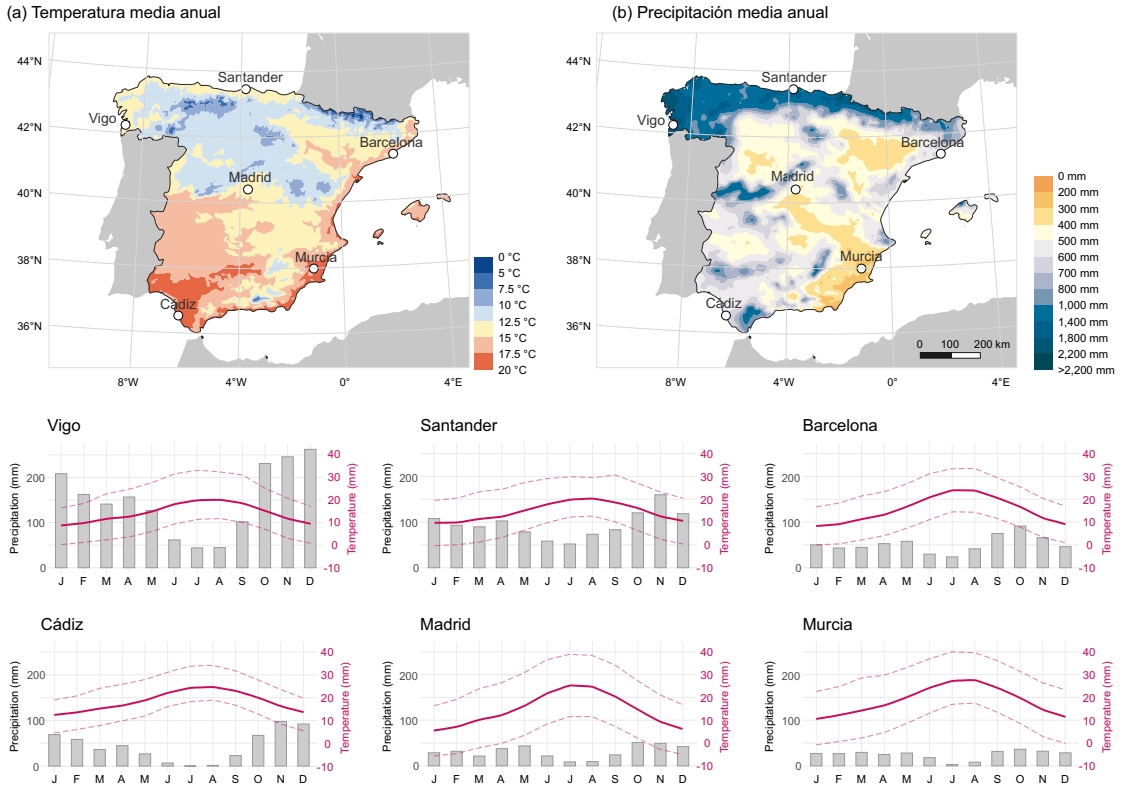


Figura 7 (a) Temperaturas medias anuales y (b) precipitaciones medias anuales en España (1981-2010). Fuente de datos: IGN (2019c). Precipitación y temperatura medias mensuales (las líneas discontinuas representan las temperaturas mínimas y máximas) en seis ciudades españolas (1981-2010). Fuente de datos: AEMET Open data (https://opendata.aemet.es).

del sur y en la depresión del Ebro (fitoclimas mediterráneos) dominan la coscoja (*Quercus coccifera*) y la encina (*Quercus ilex*). En regiones muy secas, predominan arbustos como el acebuche (*Olea europaea*) y el lentisco (*Pistacia lentiscus*; Bedia et al., 2014).

Red fluvial española y principales ríos

La red fluvial española (Figura 8) se puede dividir, de manera grosera, en las cuencas atlánticas (es decir, que vierten al océano Atlántico), que cubren alrededor del 69 % de la Península Ibérica, y las cuencas mediterráneas (es decir, que vierten al mar Mediterráneo), que cubren el 31 % restante de la Península (Ministerio de Medio Ambiente, 2004; Sabater et al., 2022).

Los contrastes climáticos, la situación geográfica y el relieve influyen profundamente en la disponibilidad de agua a lo largo de la red fluvial española (Tabla 1). Los ríos mediterráneos tienen un régimen de caudales pluvial, alcanzando su caudal máximo en primavera y otoño y mínimo en verano. Los ríos atlánticos también presentan carácter pluvial, pero tienen mayores aportaciones, con caudales máximos

a finales de invierno y estiajes menos acusados. Los ríos con régimen nival (p.e., en los Pirineos u otras cordilleras) alcanzan los niveles máximos de descarga en primavera y mantienen un caudal basal relativamente constante en verano (Sabater et al., 2022). Las cuencas mediterráneas meridionales y centrales (Guadiana, Guadalquivir, Segura y Júcar; Figura 8) cubren el 41 % del territorio, concentrando el 37 % de la población, pero cuentan sólo con el 19 % del total de los recursos hídricos disponibles. Por el contrario, las cuencas hidrográficas del norte cubren el 59 % de la superficie pero acumulan el 81 % de los recursos hídricos (Costejà et al., 2002).



Figura 8 Red fluvial española y principales cuencas hidrográficas. Fuente de los datos: MITECO (2022b) y EU-Hydro (European Environment Agency, 2015).

En el norte de la Península Ibérica, pequeños ríos de montaña discurren desde la Cordillera Cantábrica hasta el Golfo de Vizcaya. En el límite occidental de la Cordillera Cantábrica se encuentran las cuencas de los ríos Miño y Sil, que fluyen hacia el oeste hasta desembocar en el océano Atlántico (Viveen et al., 2013). Los ríos Duero, Tajo y Guadiana discurren por la Meseta y están encerrados por la Cordillera Cantábrica, el Sistema Ibérico y Sierra Morena, alcanzando el Océano Atlántico por la costa occidental. El río Ebro, el mayor curso fluvial de las cuencas mediterráneas, bordea la Meseta, llegando al mar Mediterráneo desde la costa oriental, junto con el Júcar, el Segura y otras pequeñas cuencas mediterráneas (Figura 8; Ministerio de Medio Ambiente, 2004; Sabater et al., 2022).

Tabla 1 Características generales de las mayores cuencas hidrológicas de España. Fuente: Sabater (2008) y CHMS (2022).

	Duero	Ebro	Tajo	Guadiana	Guadalquivir	Júcar	Segura	Miño-Sil
Área de cuenca (km^2)	97.290	85.362	80.600	67.048	57.527	21.208	19.182	17.581
Caudal medio anual (hm^3)	13.560	13.410	9.930	6.180	7.220	810	820	7.660
Elevación media de la cuenca (m)	874	770	599	504	566	817	644	738
Precipitación media anual (mm)	665	672	599	532	521	448	398	1,183
Temperatura media (°C)	11,4	11,4	13,8	15,2	15,5	13,1	14,4	11,4
Áreas urbanas (% de la cuenca)	0,7	0,6	1,7	0,6	1,1	0,7	1,7	1,3
Terreno cultivable	55,1	47,1	45,6	68,4	62	51,6	53,4	25,1
Pastos	1	1,9	0,3	0,1	0	0	0	2,9
Bosque	17,5	22,3	21,5	7,8	12,8	17,2	14,6	15,4
Praderas naturales	23,5	25,5	28,8	21,8	20,9	29,8	24,9	17,2
Número de grandes presas ($\geq$15 m)	75	70	72	87	55	13	15	51
Densidad de población ($habitantes.km^{-2}$)	37	34	136	24	69	207	78	45

El Duero (*Douro* en portugués) es el tercer río más largo de España (927 km en total: 597 km en España y 330 km en Portugal) y presenta la mayor cuenca hidrográfica en términos de superficie (Tabla 1; el 80 % de su superficie se encuentra en España). El clima es principalmente mediterráneo, con una influencia continental en el este y un clima atlántico, más suave, en su porción más occidental. La mayor parte de la cuenca está ocupada por ciudades de tamaño medio (León, Burgos, Valladolid; <300.000 habitantes) y pequeños pueblos (<1.000 habitantes), tierras de cultivo (trigo, avena, cebada, viñedos) concentradas principalmente en la región central), y bosques en las cabeceras de cuenca (Sabater et al., 2022).

El río Ebro es uno de los mayores ríos ibéricos (910 km), que fluye desde la Cordillera Cantábrica hasta el Mar Mediterráneo. Los contrastes climáticos e hidrológicos de la cuenca (p.e., la precipitación media anual varía entre 307 y 2.451 mm.año^{-1}) son consecuencia de una topografía muy heterogénea, caracterizada por las cadenas montañosas que rodean el valle del Ebro (López-Moreno et al., 2011). La elevada escorrentía en las cabeceras montañosas motivó la construcción de numerosas presas entre los años 1950 y 1980. Sus embalses alcanzan una capacidad de almacenamiento de unos 3.000 hm^3 (equivalente a la mitad de la escorrentía media anual de los ríos pirenaicos), que suple el 77 % de las demandas de agua para regadío y genera el 62 % de la energía hidroeléctrica producida en la cuenca del Ebro (López-Moreno et al., 2008).

El Tajo (*Tejo* en portugués) es el río más largo de la Península Ibérica y recorre 1.007 km desde la Sierra de Albarracín (centro-este de España, 1.590 m.s.n.m.) hasta su desembocadura en el Océano Atlántico, localizada en el área de Lisboa (Sabater et al., 2022). La cuenca abastece de agua a la agricultura de regadío (el principal usuario de agua, con una ocupación de 2.300 km^2), el abastecimiento urbano (aproximadamente el 15 % y el 30 % de la población española y portuguesa, respectivamente, incluidas las áreas metropolitanas de Madrid y Lisboa), la industria (refrigeración de centrales térmicas) y las actividades pesqueras en el estuario del Tajo (López-Moreno et al., 2009). Además, una parte de los caudales se transfiere a la cuenca del Segura (650 hm^3.año^{-1}; Sondermann and de Oliveira, 2022), aunque la magnitud del trasvase Tajo-Segura ha sido revisado en el tercer ciclo de los planes hidrológicos para cumplir con los requerimientos ambientales marcados por la DMA. La cuenca está muy regulada y, por ejemplo, el embalse de Alcántara (capacidad de almacenamiento de 3.162 hm^3, el segundo mayor de Europa) provoca importantes cambios en el régimen hidrológico y en los patrones de sequía del río Tajo (López-Moreno et al., 2009).

El río Guadiana está situado en el suroeste de la Península Ibérica y discurre a lo largo de 810 km (550 km en España, 150 km en Portugal; Sabater et al.,

2022). La cuenca experimenta un estrés hídrico severo debido a la influencia del clima mediterráneo (veranos calurosos y secos y precipitaciones concentradas entre octubre y abril), caracterizado por frecuentes episodios de sequía, las demandas de agua cada vez más fuertes (agricultura de regadío, turismo, usos industriales en la región de Huelva en la parte española), y la sobreexplotación de los recursos hídricos subterráneos (Bromley et al., 2001).

El río Guadalquivir (657 km de longitud) discurre principalmente por la comunidad autónoma de Andalucía. La cuenca está formada por una depresión geográfica delimitada por la Sierra Morena al norte, el Sistema Bético al sur y el Océano Atlántico al suroeste (Sabater et al., 2022). La agricultura es una importante actividad económica en la cuenca (8.560 km^2 de regadío) y el principal usuario de agua (2.741 hm^3 en 2015, correspondientes al 82 % del consumo total de agua; CHG, 2015) en la cuenca. La región es muy vulnerable al cambio climático, y la escasa disponibilidad de agua en esta región semiárida condujo a la construcción de varias presas y embalses, así como a la adopción de sistemas de riego más eficientes (Rodríguez Díaz et al., 2007; Romero-Jiménez et al., 2022).

Las cuencas del Júcar y del Segura están situadas en el este de la Península Ibérica. Los episodios de sequía son frecuentes en ambas cuencas debido al clima semiárido y a la elevada variabilidad hidrológica (Carmona et al., 2017; Miró et al., 2021). Las elevadas demandas hídricas, motivadas por la gran densidad de población (especialmente en la cuenca del Júcar, ver Tabla 1), el turismo, la agricultura intensiva de regadío (3.900 km^2 en la cuenca del Júcar y 2.624 km^2 en la cuenca del Segura), y la ganadería las hacen altamente vulnerables al estrés hídrico y a los impactos asociados al cambio climático (CHJ, 2015; CHS, 2015). Desde 1979, la cuenca del Segura recibe agua trasvasada desde la cabecera del Tajo para abastecer las demandas urbanas y agrícolas (en promedio, 350 $hm^3.año^{-1}$), lo que generó desarrollo socioeconómico en la región pero también conflictos sociales y políticos importantes asociadas al uso del agua (Senent-Aparicio et al., 2021).

Los ríos cantábricos cubren una estrecha zona del norte del país, con sus nacimientos en la Cordillera Cantábrica y desembocaduras en el mar Cantábrico. Los ríos se caracterizan por su escasa longitud, fluyendo por sus valles estrechos y encajonados y con caudales relativamente altos. Están dominados por el clima oceánico templado (inviernos suaves, veranos cálidos y precipitaciones abundantes durante todo el año), como consecuencia de su proximidad al océano y de los vientos del noroeste (Sabater et al., 2022). En total, 28 ríos principales cubren una superficie total de 23.223 km^2 (Nalón, Navia y Nervión entre las cuencas más grandes). Los usos del agua son mayoritariamente doméstico (en torno al 67 % del consumo total) e industrial (22 %), y los embalses se utilizan principalmente para la generación

hidroeléctrica a pequeña escala (CHC, 2015; CHC and URA, 2015). Los bosques, los prados y pastos para ganadería extensiva y la agricultura son los principales usos del suelo; las zonas urbanas y la industria se concentran sobre todo en la parte oriental de la costa, donde la densidad de población también es mayor (Díez et al., 2000; Prego et al., 2008).

La cuenca del Miño-Sil ocupa una superficie relativamente pequeña en el noroeste del país, pero presenta un caudal medio anual por encima de la media nacional (Tabla 1). El clima tiene una fuerte influencia atlántica, con temperaturas suaves y precipitaciones abundantes durante todo el año (entre 700 y 1.900 mm.año^{-1}). El sector agropecuario es el principal usuario de agua (el 67 % se destina al riego y el 6,4 % a la ganadería), seguido por el consumo urbano (16,1 %) y el industrial (8,4 %). La cuenca está regulada por más de 70 grandes presas (>10 m), con Belesar (654,1 hm^3), As Portas (535,7 hm^3), Barcena (340 hm^3) entre las más grandes (CHMS, 2022).

Por último, la red fluvial española se completa con una serie de cuencas de menor entidad. Son las denominadas cuencas intracomunitarias (es decir, que fluyen íntegramente dentro de una comunidad autónoma), y que incluyen las Cuencas Mediterráneas Andaluzas (17.762 km^2; Junta de Andalucia, 2015), las Cuencas Internas de Cataluña (16.438 km^2; Agència Catalana de l'Aigua, 2022) y las cuencas de Galicia Costa (12.991 km^2; Augas de Galicia, 2023).

Usos del agua en España

El consumo de agua para uso agrícola y ganadero suponen el 78,8 % del consumo total del país, mientras que el abastecimiento urbano y los usos industriales consumen el 17,3 % y 3,9 %, respectivamente (Fornés et al., 2021). Con una superficie de regadío de 38.779 km^2 en el país (MAPAMA, 2021), el consumo de agua para riego es extremadamente elevado (estimado en 15.495 hm^3 en 2018, de los que casi el 75 % procede de aguas superficiales; INE, 2020) y se concentra mayoritariamente en las cuencas mediterráneas (Figura 9). Los regadíos suponen el 60 % de la producción agraria total y el 80 % de las exportaciones agrarias del país, representando una actividad económica esencial en España (Berbel et al., 2019; MAPAMA, 2021).

La captación de agua subterránea (alrededor de 7.000 hm^3.año^{-1}) corresponde al 22 % de la demanda total de agua en España y abastece de agua a unos 13 millones de habitantes (28 % de la población) y alrededor del 27 % de la superficie total de regadío (De Stefano et al., 2015). Los porcentajes más elevados de captación de aguas subterráneas en relación con la captación total de la cuenca hidrográfica se dan en cuencas de Canarias y Baleares, Júcar, Cataluña y Andalucía (De Stefano et al.,

2012; Figura 9). En muchas regiones, las aguas subterráneas son un recurso hídrico estratégico para suplir la demanda de consumo humano, especialmente durante las épocas de estiaje y sequías cuando el recursos en forma de agua superficial es realmente escaso, por ejemplo, para abastecer a la población de Madrid (Béjar-Pizarro et al., 2017). Sin embargo, el principal uso de las aguas subterráneas se da, una vez más, en el sector agrícola (De Stefano et al., 2015). La gestión de los recursos hídricos subterráneos es una tarea compleja y algunos de los acuíferos, especialmente en el sur del país, están actualmente sobreexplotados (Fornés et al., 2005). Por ejemplo, en la cuenca del Guadiana, la sobreexplotación de los recursos hídricos subterráneos desde la década de 70 para el regadío ha provocado una reducción crónica del nivel freático y de los recursos hídricos, causando la degradación de los ecosistemas fluviales y de humedales (Bromley et al., 2001).

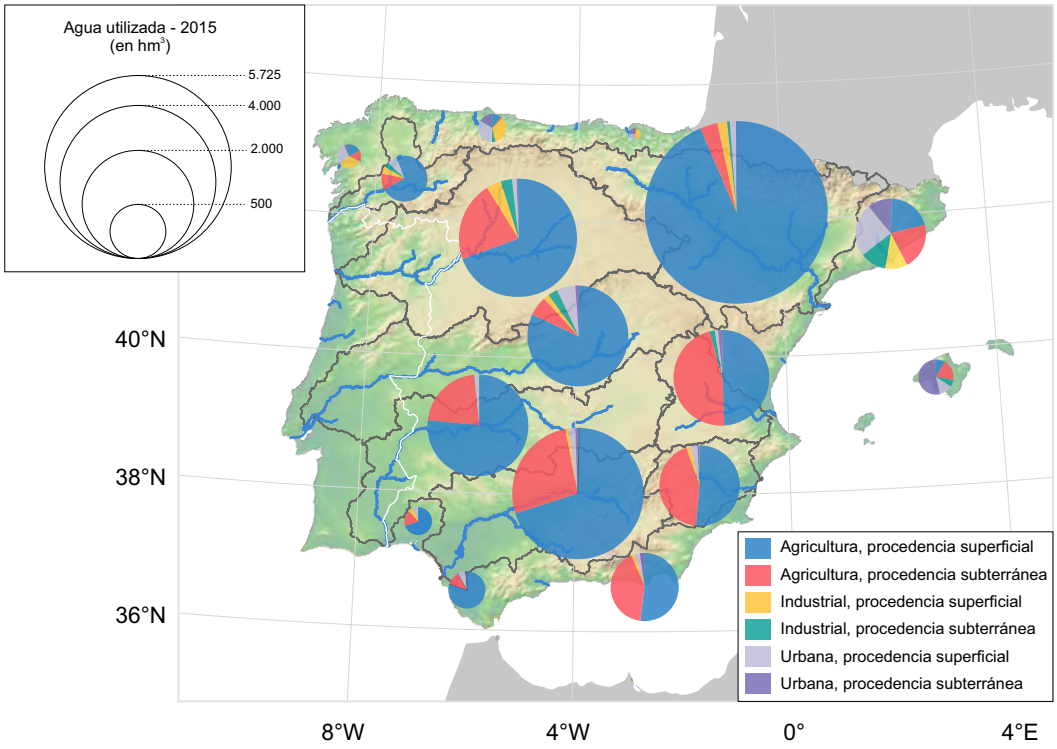


Figura 9 Usos del agua en 2015 en las principales cuencas hidrográficas españolas. Adaptado de IGN (2019a).

Ante esta situación y el desarrollo de la actividad agrícola, la construcción de presas y embalses aumentó rápidamente en los ríos ibéricos durante el siglo XX y, durante más de tres décadas tras la Guerra Civil española (1936-1939), el régimen franquista promovió su construcción. En 1940, los embalses en España tenían una capacidad total de almacenamiento de 4.000 hm^3; en 1975, esta capacidad aumentó a 37.000 hm^3 , con más de 800 presas (Fornés et al., 2021).

Hoy en día, a pesar de ser un país relativamente pequeño, España se encuentra entre los mayores constructores de presas del mundo, con 1.064 grandes presas (altura ≥15 m o embalse de más de 3 hm^3; ICOLD, 2021). La escorrentía total del país se estima en unos 111.000 hm^3 al año, y la capacidad total de los embalses es de 56.069 hm^3 (MITECO, 2022a), con La Serena (3.220 hm^3 capacidad, río Zújar), Alcántara (3.162 hm^3, río Tajo), y La Almendra (2.649 hm^3, río Tormes en la cuenca del Duero) como los mayores embalses de España (Figura 10; IGN, 2019a).

El porcentaje de almacenamiento de agua en el país es esencial para atender las demandas actuales dadas las condiciones climáticas secas y a la irregularidad de las precipitaciones (Fornés et al., 2021). Las presas aumentan significativamente la disponibilidad de agua en las cuencas meridionales de España, atenuando el impacto de la reducción del caudal de los arroyos y las incertidumbres de las proyecciones del cambio climático (Granados et al., 2021). Por ejemplo, en la cuenca del Guadiana, el embalse de La Serena (el mayor de España y el tercero de Europa, véase la Figura 10), aumentó el volumen de agua almacenada más de 40 veces respecto al obtenido en condiciones de caudal natural (Ministerio de Medio Ambiente, 2004).

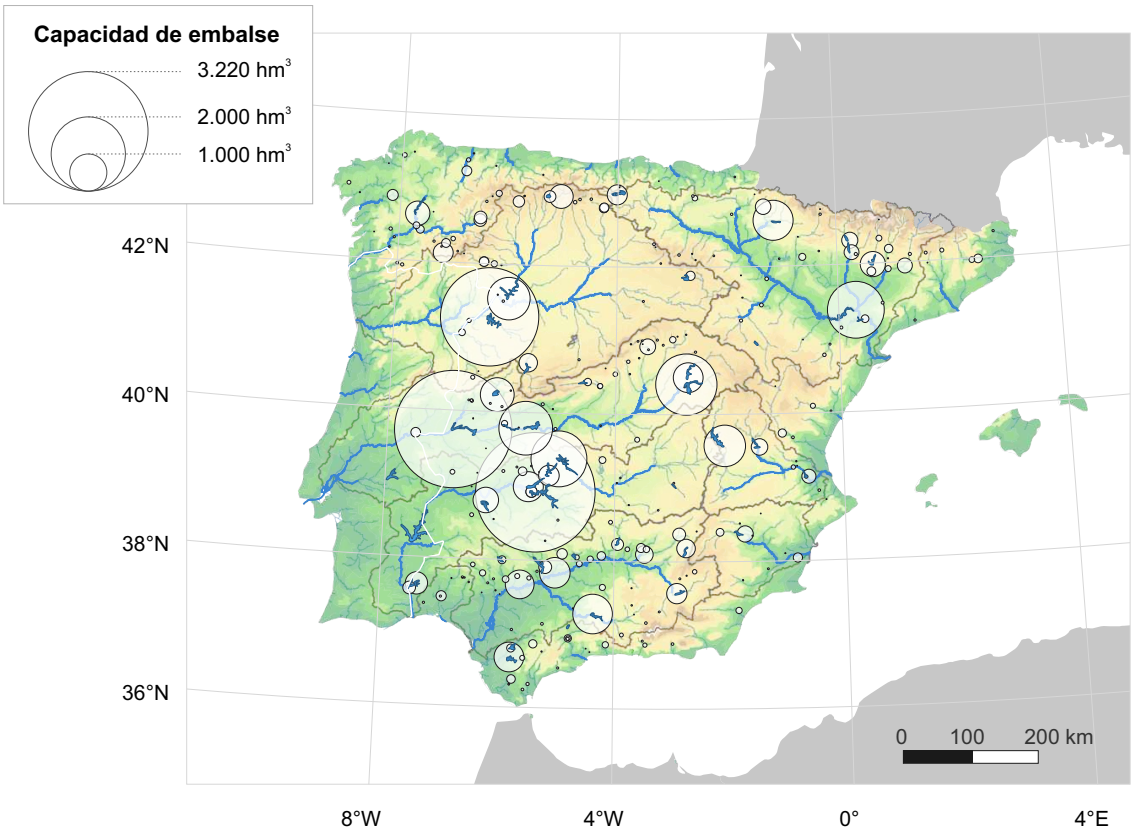


Figura 10 Distribución de los principales embalses de España y su capacidad. Adaptado de IGN (2019a).

La construcción y la operación de presas provoca cambios en la estacionalidad de los caudales, la variabilidad intraanual y los caudales mínimos y máximos en

ríos españoles (Peñas and Barquín, 2019). En general, las presas provocan una menor variabilidad de caudales durante la estación húmeda, con un aumento de la frecuencia de eventos de caudal bajo y una disminución de la frecuencia de eventos de caudal alto. Durante la estación seca ocurre lo contrario, es decir, la variabilidad del caudal aumenta, los eventos de caudales bajos son menos frecuentes, mientras que los caudales altos son más frecuentes (Mezger et al., 2021). Sin embargo, los patrones de alteración de caudales también dependen de las condiciones hidrológicas naturales, es decir, de los regímenes de caudales previos a la construcción presa (Peñas and Barquín, 2019).

Estado actual de los ríos españoles y la gestión de caudales ecológicos

En 1985, con la Ley de Aguas en España, la elaboración de planes hidrológicos de cuenca se convirtió en un requisito legal (Willaarts et al., 2014). Posteriormente, con la trasposición de la DMA a la legislación española en 2003, la conservación de los ecosistemas acuáticos se convirtió en una de las prioridades de la política de aguas. Las confederaciones hidrográficas y las agencias del Agua se encargaron entonces de elaborar los nuevos planes hidrológicos, donde se fijan los objetivos de protección ambiental, y se establecen las medidas de gestión los recursos hídricos superficiales y subterráneos y las infraestructuras (p.e., presas y canales), y se definen los programas de seguimiento del régimen de caudales, la calidad y el estado ecológico de las masas de agua (Font and Subirats, 2010; Román-Barreiro, 2018).

Como parte de la DMA, las masas de agua se agruparon en función de sus atributos físicos y morfológicos (p.e., altitud, conductividad del agua, geología y tamaño de la cuenca) en tipologías ecológicas, lo que contribuyó a realizar evaluaciones del estado de las masas de agua de forma más específica y precisa (Hering et al., 2010). Los ríos españoles se clasificaron en un total de 37 tipologías fluviales (p.e., ríos mediterráneos kársticos, ríos mediterráneos costeros, ríos de montaña silíceos húmedos, ejes fluviales cantábrico-atlánticos silíceos) y se establecieron, para cada una de ellas (CEDEX, 2004; BOE, 2005), sus condiciones biológicas, hidromorfológicas y químicas de referencia (es decir, condiciones basadas en lugares con cambios mínimos y ausencia de presiones humanas significativas; Wallin et al., 2003). Este paso supuso un gran reto, dada la alta variabilidad ambiental, el alto grado de antropización de los ríos españoles y el concepto poco claro de lo que representaban las condiciones mínimamente alteradas dentro de la DMA (Pardo et al., 2012).

Tras el primer ciclo de planificación hidrológica (2009-2015), los segundos planes hidrológicos de cuenca (2016-2021) indicaban que el 51 % de las aguas superficiales

de España se encontraban en buen o muy buen estado ecológico (MITECO, 2018). Los ríos tenían el 55 % de sus masas de agua clasificadas en estado ecológico bueno o muy bueno. La situación era más crítica en los lagos y humedales y en las aguas de transición, ya que sólo el 45 % y el 46 % de las masas de agua, respectivamente, se encontraban en un estado ecológico bueno o superior. Por el contrario, las aguas costeras, que corresponden a las aguas situadas hasta una milla náutica de la línea de costa, tenían el 78 % de sus masas de agua en estado ecológico bueno o superior (European Commission, 2000; European Environment Agency, 2018b).

El estado ecológico general de las aguas superficiales presenta una gran variabilidad a lo largo del país (Figura 11). En general, las zonas más problemáticas eran las cuencas del Duero (79 % de las masas de agua en mal estado), el Guadiana y Cataluña. Las masas de agua del norte (es decir, las cuencas cantábricas, gallegas y la del Miño-Sil) presentaron el mejor estado ecológico (en torno al 70 % de las masas de agua en buen estado; Willaarts et al., 2014). Sin embargo, estos datos deben interpretarse con cautela, ya la mayoría de las evaluaciones ecológicas se basaron únicamente en macroinvertebrados y diatomeas (comunidades de peces evaluadas en el 8 % de las masas de agua; continuidad fluvial en el 18 %; solo dos de las 17 cuencas han desarrollado métricas y condiciones de referencia para estos elementos; European Environment Agency, 2018b; Willaarts et al., 2014).

Por otro lado, la legislación española en materia de aguas (ARM/2656/2008; BOE, 2008) exige el establecimiento de caudales ecológicos como parte de los planes hidrológicos de cuenca, siendo su principal objetivo el mantenimiento de la integridad de los ecosistemas acuáticos aguas abajo de las presas. Esta normativa contemple la definición de caudales ecológicos mínimos y máximos y de su distribución temporal, su tasa de variación y exige el establecimiento de un régimen de eventos de caudales de crecidas (es decir, episodios de corta duración, con magnitud equivalente al caudal de *bankfull*). Los caudales ecológicos mínimos y máximos se determinan combinando métodos de simulación hidrológica y de hábitats (normalmente para especies piscícolas en dos estadios diferentes; BOE, 2008).

Sin embargo, pese a la entrada en vigor de esta normativa, la implementación de caudales ecológicos en el país ha considerado eminentemente los caudales mínimos (73 % de las masas de agua), mientras que el resto de elementos se han implementado en menos del 8 % de las masas de agua (Mezger et al., 2019). Los eventos de caudales de crecida y la tasa de cambio son variables clave para mantener la integridad de los ecosistemas acuáticos (Bunn and Arthington, 2002; Poff and Zimmerman, 2010). Los pulsos de caudal alto y las crecidas de mayor envergadura determinan las características hidromorfológicas del canal fluvial y del sustrato del lecho, restablecen la calidad del agua tras periodos prolongados de bajo caudal,

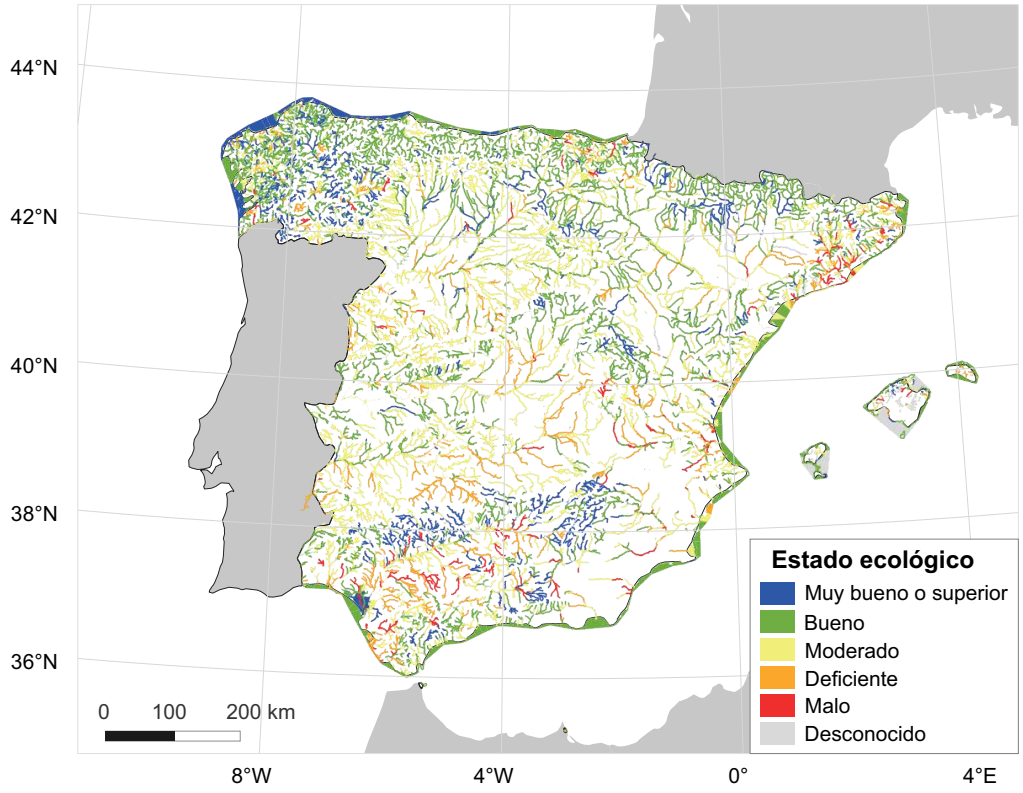


Figura 11 Visión general de las masas de agua en España y su estado ecológico en los segundos planes hidrológicos de cuenca (2015-2021). Fuente de datos: MITECO (2016).

arrastran los contaminantes y las especies invasoras y mantienen la conectividad fluvial (p.e., la conectividad lateral con las llanuras aluviales; Richter and Thomas, 2007).

Objetivos y estructura de la tesis

El objetivo principal de esta tesis doctoral es evaluar los patrones de variabilidad espacial de la biodiversidad en los ríos españoles y cómo las presas y embalses modifican estos patrones. Para ello, es necesario separar la variabilidad de la biodiversidad derivada de gradientes y factores naturales de la variabilidad asociada a factores antrópicos (es decir, las presas).

Esta tesis consta de seis capítulos. La Introdución aborda los temas clave relacionados con los ecosistemas de agua dulce y en especial los ríos, su biodiversidad y sus amenazas, los efectos de las presas en el régimen de caudales y las medidas de mitigación, entre otros temas relevantes. Los capítulos 2 a 5 (Figura 12) exploran los objetivos específicos que se derivan de las cuestiones y lagunas de investigación identificadas en la Introducción. Por último, las Conclusiones se exponen las

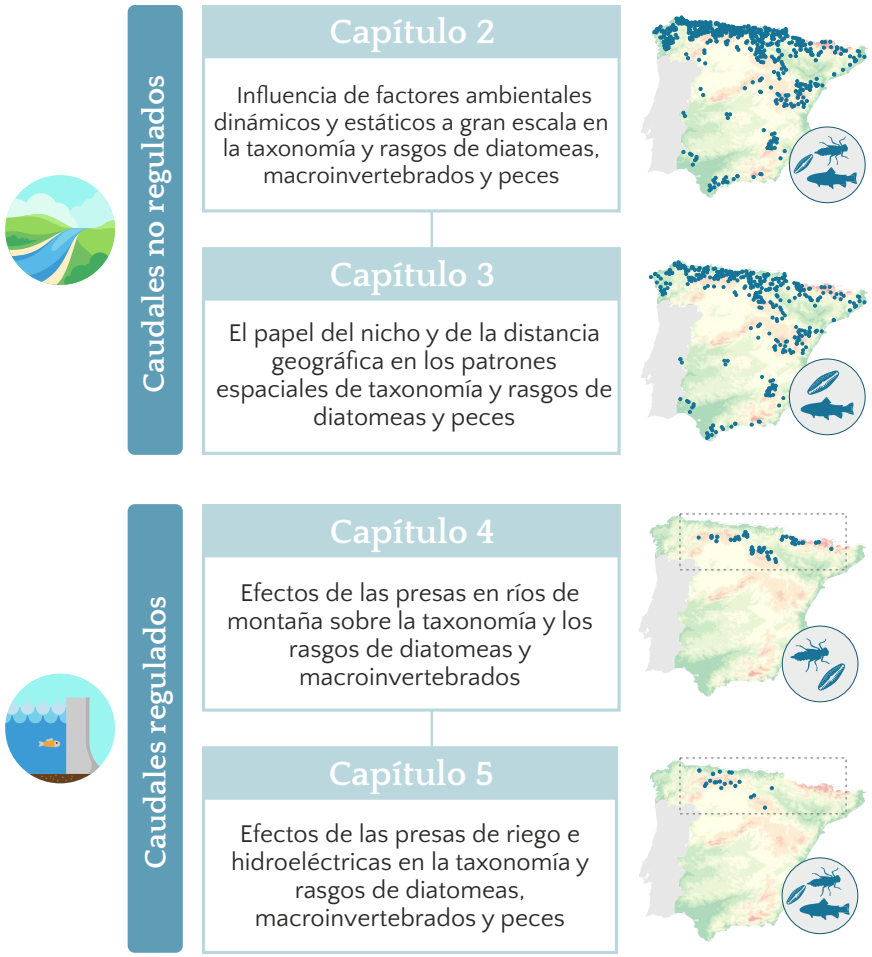


Figura 12 Los cuatro capítulos principales de la tesis, que exploran las comunidades biológicas de agua dulce en regímenes de caudal natural y regulado. Los mapas de la derecha indican la distribución espacial de las muestras en cada capítulo.

conclusiones generales derivadas de los capítulos previos y las futuras líneas de investigación, seguidas de Referencias y Anejos A y B.

Más concretamente, los capítulos 2 y 3 tienen como objetivo explorar la variabilidad natural de las comunidades biológicas de los ecosistemas fluviales, considerando grandes escalas espaciales. Por su parte, los capítulos 4 y 5 exploran cómo presas con diferentes fines, usos y esquemas de explotación, afectan a las comunidades biológicas, basándose en los conocimientos adquiridos en los dos capítulos anteriores. Por lo tanto, los objetivos específicos se exploran en los siguientes capítulos de la tesis:

- Evaluar la contribución de los factores abióticos (p.e., ambientales e hidrológicos) en la variabilidad de la comunidad de diatomeas, macroinvertebrados y peces en ríos prístinos con condiciones de caudal natural distribuidos a lo largo de España (Capítulo 2).

- Investigar la contribución de las variables locales (es decir, el nicho) y espaciales (es decir, los procesos relacionados con la dispersión) en la estructura de las comunidades de diatomeas y peces en ríos prístinos con condiciones de caudal natural distribuidos a lo largo de España (Capítulo 3).

- Evaluar el impacto de las presas en las comunidades de diatomeas, macroinvertebrados y peces e identificar los principales impactos producidos por cambios en factores hidrológicos y fisicoquímicos en ríos de montaña (Capítulo 4) y en un diseño de estudio de control-impacto (Capítulo 5) en el norte de España.

- Comparar los efectos de la regulación del caudal sobre la biota fluvial asociados a las presas gestionadas para el riego y la generación de energía hidroeléctrica (Capítulo 5).

- Comparar el uso de múltiples grupos bióticos (es decir, diatomeas, macroinvertebrados y peces) como bioindicadores en términos de sensibilidad a la alteración hidrológica y a factores ambientales, a procesos de dispersión y a la escala espacial.

- Evaluar las ventajas e inconvenientes de la utilización de los rasgos de las especies en el contexto de la monitorización mediante bioindicadores y la conservación de los ecosistemas de agua dulce.

Capítulo 2: Evaluación de los factores determinantes en la distribución de las comunidades biológicas a gran escala: una visión de los efectos del cambio global en los ecosistemas fluviales

El cambio global al que se ven sometidos los ecosistemas actualmente supone una grave amenaza para los ríos. En este contexto, aumentar nuestro conocimiento sobre cómo los factores de estrés afectan a las comunidades biológicas es fundamental para apoyar estrategias de gestión y conservación eficaces. En este capítulo hemos evaluado los principales gradientes ambientales que influyen en la variabilidad espacial de la biodiversidad de ríos en la España continental, utilizando variables ambientales a escala de paisaje que representan los gradientes de clima, usos y ocupación del suelo (LULC), regímenes de caudal, geología y topografía. Se han utilizado comunidades de diatomeas (n=117), macroinvertebrados (n=441) y peces (n=264) muestreadas en tramos fluviales casi prístinos (Figura 13). Se utilizó el análisis de redundancia para identificar los factores ambientales que contribuían significativamente a la variabilidad de las tres comunidades, y el análisis RLQ para evaluar las asociaciones entre la distribución de rasgos y su relación con las variables ambientales.

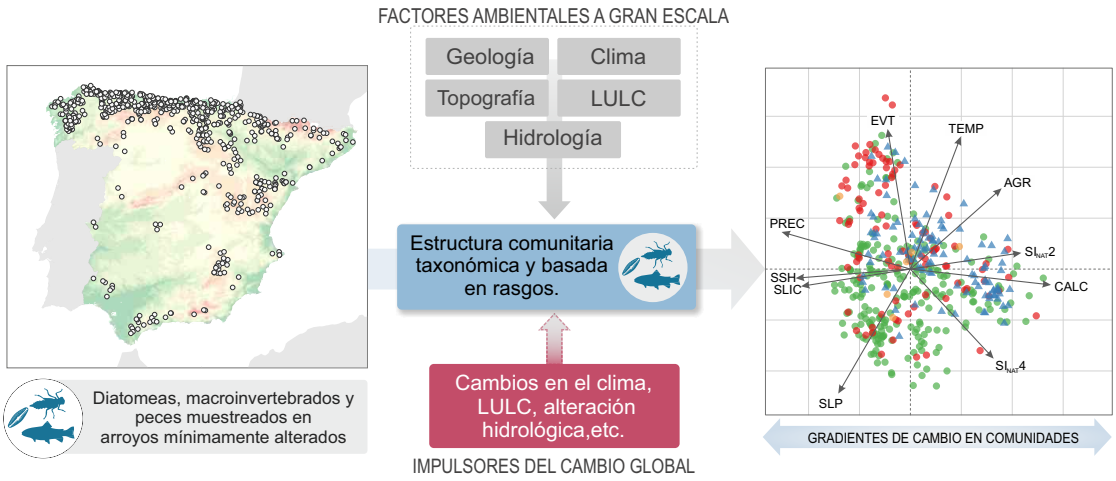


Figura 13 Mapa de los puntos de muestreo, factores medioambientales a gran escala considerados en el estudio que pueden estar asociados a los impulsores del cambio global y respuestas rasgo-medio ambiente.

Los cuatro grupos de variables ambientales consideradas contribuyeron a identificar los principales gradientes de cambio de las comunidades biológicas (p.e., cabeceras vs tramos bajos). Los ríos silicios, con pendientes fuetes y niveles de precipitaciones elevados favorecieron comunidades dominadas por diatomeas pedunculadas, macroinvertebrados con estrategias de dispersión acuática pasiva y

especies piscícolas migradoras. Estos rasgos fueron sustituidos por diatomeas adnatas, pioneras y móviles, macroinvertebrados de pequeño tamaño, no insectos y peces no migratorios en los tramos bajos, dominados por climas más cálidos, geología calcárea, con mayor proporción de suelo agrícola en la cuenca y regímenes de caudal estables.

En general, las variables ambientales a escala de paisaje mostraron una contribución significativa a la variabilidad de las comunidades de peces, mientras que esta fue menor en el caso se las diatomeas y los macroinvertebrados, que podrían estar influenciadas en mayor grado por otras variables a escala loca (p.e., la calidad del agua). El cambio gradual de las variables ambientales a lo largo de las redes fluviales (p.e., cambios en la pendiente, temperatura, usos humanos del suelo) generó cambios análogos tanto en la variabilidad espacial de la taxonómica como de los rasgos en los tres grupos bióticos.

Estos resultados indican que los efectos del cambio global podrían generar cambios en la distribución longitudinal de las comunidades fluviales a lo largo de las redes fluviales y que, por tanto, la aplicación de medidas de gestión que consideren de manera conjunta los cambios de la temperatura del agua y de régimen hidrológico (p.e., abordando los cambios en LULC) podría suponer una estrategia idónea para mitigar los efectos del cambio global en los ríos.

Capítulo 3: Evaluación de los patrones de variación espacial a gran escala de las comunidades fluviales: Nicho versus distancia geográfica

Este capítulo es una versión editada del artículo de investigación publicado en la revista Biodiversity and Conservation por Cássia Rocha Pompeu, Francisco J. Peñas y José Barquín en 2022 con el título "*Large-scale spatial patterns of riverine communities: niche versus geographical distance*". DOI: 10.1007/s10531-022-02514-6.

Los ecosistemas de agua dulce se encuentran entre los más amenazados de la Tierra. La definición de estrategias de conservación eficaces, basadas en un conocimiento sólido de los patrones de biodiversidad y de los principales factores que los estructuran, son más necesarios que nunca para revertir esta tendencia. En el capítulo 2, hemos investigado y evaluado el peso de los factores ambientales y de los factores de dispersión-conectividad a la hora de determinar la variabilidad de las comunidades de diatomeas y peces de agua dulce. Para ello, hemos utilizamos 441 muestras biológicas obtenidas de diferentes programas de monitorización biológica en ríos españoles, cubriendo un gradiente ambiental muy variable a lo largo de la red fluvial nacional. A partir de esos datos, hemos comparado la dependencia espacial de la diversidad taxonómica y de rasgos de los dos grupos bióticos mediante el análisis de la relaciones de distancia-decaimiento de la similitud biológica y de la partición de la variación con aleatorizaciones espacialmente restringidas.

Los resultados indicaron que la mayor parte de la variación biológica de las diatomeas y los peces se puede atribuir a la variación puramente espacial y espacialmente estructurada de las características ambientales. En comparación con las diatomeas, la composición de la comunidad de peces presentó una mayor dependencia espacial, probablemente debido a su menor capacidad de dispersión, restringida al propio río (Figura 14). Además, las características ambientales mostraron una mayor capacidad de predicción de la variación de las comunidades de peces. Las similitudes basadas en los rasgos presentaron una menor dependencia espacial que la similitud taxonómica entre ríos, lo que indica que son menos susceptibles a los efectos de dispersión-conectividad.

Estos hallazgos contribuyen a comprender los mecanismos subyacentes a los patrones de biodiversidad de las comunidades fluviales cuando se consideran grandes escalas espaciales (es decir, dentro y fuera de la red fluvial). Nuestros resultados ponen de manifiesto la importancia de los procesos de dispersión-conectividad a la hora de entender esos patrones, pese a lo cual son ignorados muchas en los

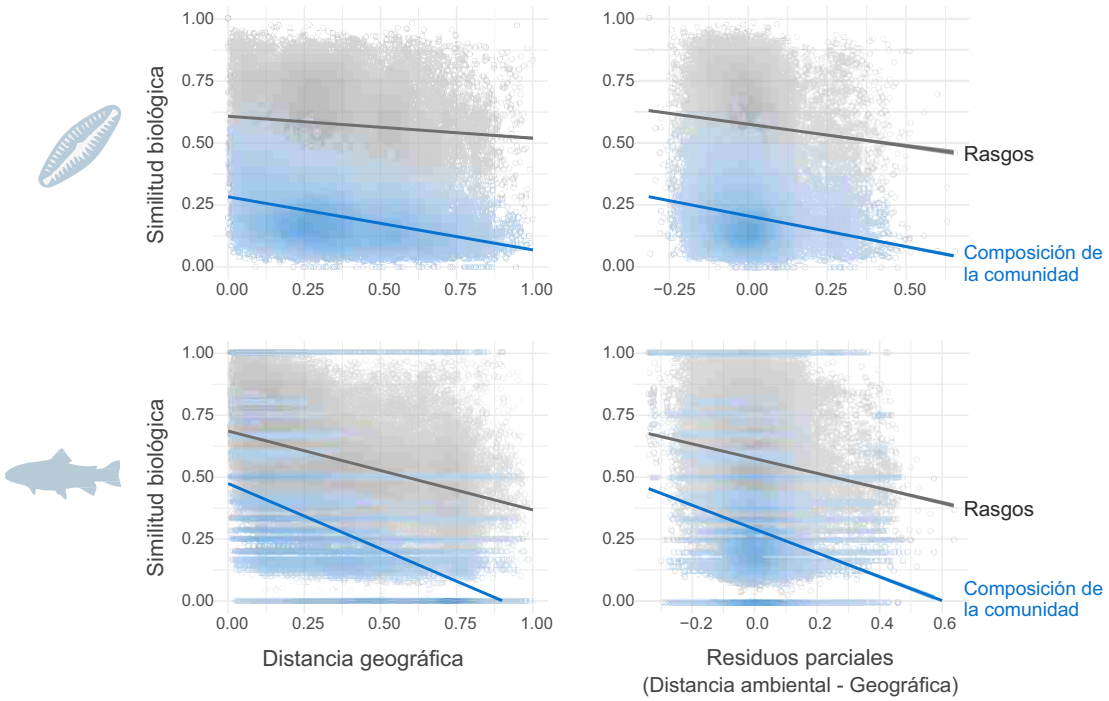


Figura 14 Relaciones de decaimiento de la similitud biológica con la distancia geográfica y con las distancias medioambientales (tras eliminar los efectos de la distancia geográfica) en comunidades de diatomeas y peces.

programas tradicionales de monitorización biológica, enmascarando la detección de impactos antropogénicos. Por lo tanto, la integración del componente de dispersión-conectividad, así como la información sobre la capacidad de dispersión de los organismos, son cruciales a la hora de establecer objetivos de conservación eficaces y diseñar estrategias de biomonitorización.

Capítulo 4: Impactos de las presas de uso agrícola sobre las comunidades biológicas en ríos de montaña

Los ríos de montaña albergan una biodiversidad única y proporcionan servicios ecosistémicos esenciales a las sociedades. Sin embargo, la integridad de estos delicados ecosistemas se ve amenazada a numerosas presiones, entre las que destacan destacan la construcción de presas y los cambios en el uso del suelo, que han provocado una rápida degradación del hábitat, la contaminación del agua y la pérdida de biodiversidad. En este capítulo, hemos evaluado el efecto de las presas con fines agrícolas sobre la biota de ríos de montaña. La evaluación se ha fundamentado tanto en el cambio en los índices bióticos tradicionales como en el cambio sufrido por las comunidades biológicas desde un enfoque basado en rasgos.

En este estudio, hemos seleccionado comunidades de diatomeas y macroinvertebrados, muestreadas entre 2015 y 2017, en 23 tramos fluviales situados en tres sistemas montañosos del norte de España (Cordillera Cantábrica, Sistema Ibérico y Pirineos). Los tramos fluviales seccionados se localizan tanto aguas abajo de presas de usos agrícola, es decir, sitios impactados (n=11), como en tramos con condiciones de caudal natural, es decir, sitios control (n=12; Figura 15). Se utilizó un análisis de la varianza de dos vías (ANOVA; factores: presa y cordillera) para determinar (a) los efectos generales de la presencia de presas (control vs. sitios alterados), (b) los efectos generales asociados a la región (Cordillera Cantábrica, Sistema Ibérico y Pirineos), y (c) las posibles interacciones entre la presencia de presas y la región sobre la hidrología (a través de un conjunto de índices hidrológicos), la fisicoquímica del agua y las comunidades biológicas.

Los resultados mostraron que las presas de uso agrícola consideradas en este estudio modificaban significativamente magnitud de los caudales a lo largo de todo el ciclo anual (verano: julio, agosto y septiembre; invierno: diciembre; primavera: Marzo), la frecuencia de los episodios de caudal alto y la temporalidad de los caudales mínimos, independientemente de la región donde se localizasen, mientras que las cordilleras mostraban diferencias significativas en cuanto a diferencias la frecuencia y duración de los eventos de caudal altos y bajos. La interacción entre el efecto de las presas y la región también produjo cambios significativos en los caudales de la época invernal y estival, la magnitud de caudales de avenida y el número de días con incremento y descenso de caudal (tasa de cambio).

En este sentido, en la Cordillera Cantábrica, una región con embalses relativamente grandes (>150 hm^3), los lugares impactados mostraron una marcada inversión de los patrones de caudal estacional (es decir, aumentos de los caudales estivales

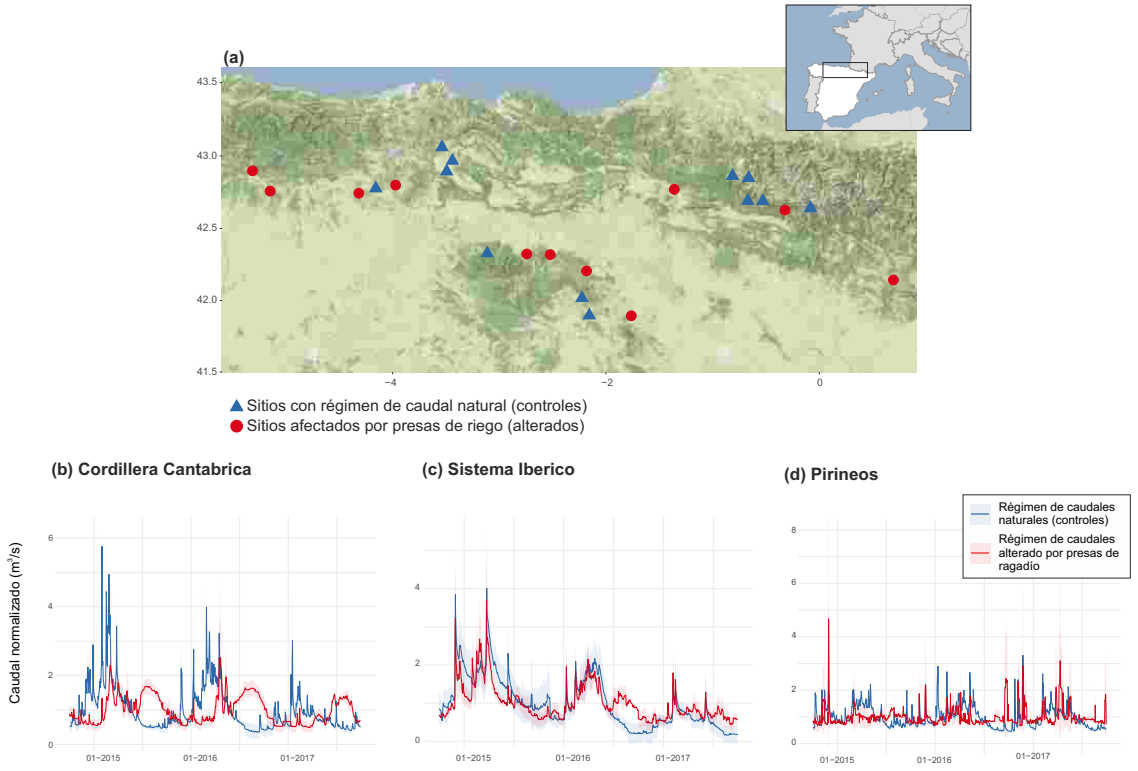


Figura 15 (a) Localización de los 23 ríos estudiados: controles y sitios afectados por presas de regadío. Caudal medio diario normalizado entre 2015 y 2017 en controles y sitios afectados por presas de regadío en (b) la Cordillera Cantábrica, (c) el Sistema Ibérico y (d) los Pirineos.

pero reducción de los caudales invernales). En las demás cadenas montañosas, los embalses presentaban menor capacidad de almacenamiento y, además, en muchos casos no tenían un fin único, lo que provocó variaciones significativas únicamente en la las tasas de cambio. Los rasgos de las diatomeas, la riqueza taxonómica, la diversidad y la puntuación del índice IPS variaron con la presencia de la presa y la cordillera; los rasgos de los macroinvertebrados y los índices bióticos no respondieron significativamente a la regulación del caudal. Esto sugiere que las comunidades de diatomeas podrían ser más sensibles a la alteración hidrológica, mientras que los macroinvertebrados podrían estar más influidos por procesos relacionados con el espacio (p.e., la dispersión) y la biogeografía, que por los propios efectos de la presa.

Nuestros resultados ponen de manifiesto la potencialidad de las bases de datos existentes con coberturas espaciales amplias para mejorar nuestro conocimiento sobre los efectos de las presas y otras presiones antrópicas. Aunque la heterogeneidad y la calidad de esas bases de datos conlleva ciertas limitaciones que tienen que ser consideradas, los resultados de este capítulo indican que pueden servir de base para definir futuras estrategias de monitorización para cuantificar el impacto asociado a la regulación de caudales y promover, en una fase posterior, una gestión sostenible de las infraestructuras y otras estrategias de conservación.

Capítulo 5: Evaluación de los efectos del riego y de las presas hidroeléctricas en las comunidades fluviales mediante enfoques taxonómicos y basados en rasgos

Este capítulo se ha publicado en la revista Ecological Indicators (145) por Cássia Rocha Pompeu, Francisco J. Peñas, Alejandra Goldenberg-Vilar, Mario Álvarez-Cabria y José Barquín en 2022 con el título "*Assessing the effects of irrigation and hydropower dams on river communities using taxonomic and multiple trait-based approaches*". DOI: 10.1016/j.ecolind.2022.109662.

Los ríos y arroyos de todo el mundo han sido y son modificados mediante diferentes tipos de infraestructuras para atender la creciente demanda de agua. Entre ellas, las presas y los embalses causan algunos de los impactos ecológicos más graves y severos en los ríos, alterando los regímenes hidrológicos y térmicos, los flujos de nutrientes y sedimentos y disminuyendo la conectividad a lo largo de la red. Sin embargo, el conocimiento de los impactos de estas infraestructuras sobre las comunidades biológicas de los ecosistemas fluviales sigue siendo limitado, especialmente si consideramos los diferentes usos y esquemas gestión de las presas.

El principal objetivo de este capítulo era evaluar los efectos de las presas (p.e., la alteración hidrológica y térmica y cambios en la calidad del agua) sobre las comunidades biológicas e identificar las respuestas ecológicas clave asociadas a la regulación del caudal. Para cumplir este objetivo, se estudiaron y monitorizaron las comunidades de diatomeas, macroinvertebrados y peces en ríos naturales (n=8), es decir controles, e impactados por presas de regadío o hidroeléctricas (n=11) a lo largo de tres años consecutivos (2017-2019). El diseño del estudio pretendía minimizar la variabilidad ambiental entre los tramos control y los impacto utilizando una clasificación hidrológica previamente establecida. Esto permitió centrarse principalmente en los efectos de los esquemas de explotación de las presas. Además de los índices bióticos tradicionales basados en la composición y estructura de estas comunidades, se evaluó las respuestas a nivel de comunidad mediante análisis basados en rasgos, utilizando en ambos casos análisis multivariantes.

Los cambios observados variaron en función de la finalidad de la presa y, en general, favorecieron las comunidades dominadas por rasgos tolerantes a las perturbaciones. Los índices bióticos tradicionales no respondieron de forma consistente al tipo de uso de las presas; los análisis basados en rasgos, por el contrario, proporcionaron una imagen más detallada de los efectos de las presas sobre las comunidades. En

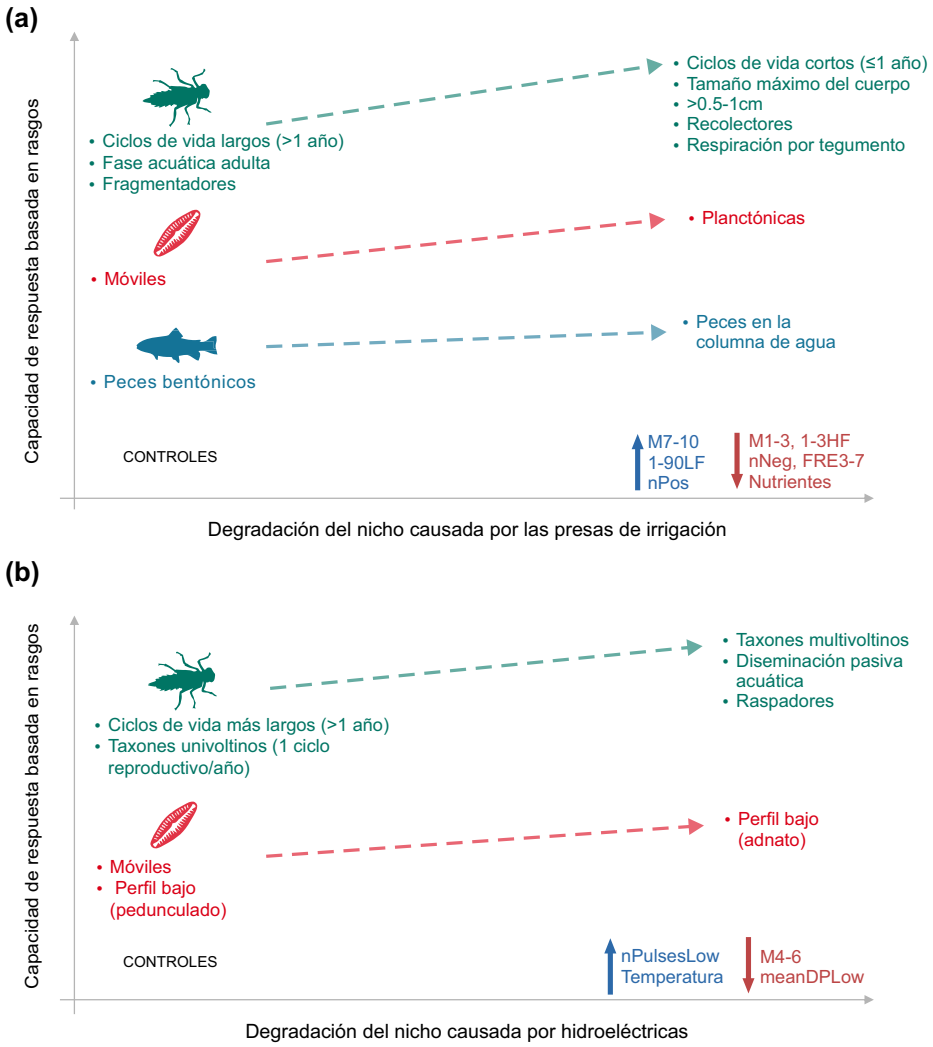


Figura 16 Principales cambios en los rasgos dominantes de las comunidades en diatomeas (en rojo), macroinvertebrados (en verde) y peces (en azul) asociados a los cambios inducidos por (a) presas de regadío y (b) presas hidroeléctricas.

este contexto, las comunidades de macroinvertebrados, analizadas en términos de sus rasgos dominantes, mostraron las correlaciones más fuertes con as variables hidrológicas y fisicoquímicas, seguidos de las diatomeas y los peces.

La inversión de los regímenes de caudal estacional (p.e., aumentos de los caudales estivales y de la magnitud de los caudales bajos extremos; descensos de los caudales invernales y de la frecuencia y magnitud de los episodios de caudales altos extremos) y la reducción de la concentración de nutrientes, generaron los cambios más significativos en las comunidades biológicas aguas abajo de las presas de riego. Estos cambios favorecieron el incremento de diatomeas planctónicas, macroinvertebrados con ciclos de vida corto y tamaño corporal reducido, y especies de peces que se alimentan en la columna de agua. Aguas abajo de las presas hidroeléctricas se encontraron comunidades dominadas por diatomeas adnatas de bajo perfil, y macroinvertebrados

multivoltinos, de dispersión pasiva y rascadores (Figura 16), lo que se relacionó significativamente con los cambios en la tasa de cambio del caudal y la temperatura del agua.

Las relaciones identificadas en este estudio resultan de una importancia crítica para apuntalar futuras estrategias de conservación encaminadas a reducir los efectos negativos de las presas y para definir nuevas líneas de investigación que ayuden a comprender como estas infraestructuras modifican el ecosistema fluvial.

Conclusiones generales

El objetivo principal de esta tesis fue evaluar los patrones de variabilidad espacial de la biodiversidad en los arroyos y ríos españoles y cómo las presas y embalses modifican estos patrones. Este objetivo se abordó desde diferentes perspectivas (p.e., a escala de cuenca y a escala espacial nacional, en regímenes naturales y regímenes alterados, y considerando presas con diferentes tipos de finalidad y esquema de operación). Además, el análisis de múltiples grupos biológicos que representan diferentes niveles tróficos en el ecosistema fluvial, desempeñó un papel clave en este trabajo de investigación, proporcionando una visión útil sobre cómo comunidades específicas podrían verse perjudicadas por las presas y, por lo tanto, cuáles podrían ser las siguientes direcciones para mejorar su gestión y aminorar los impactos.

Influencia de los factores ambientales y espaciales en la distribución natural de las comunidades biológicas

En el Capítulo 2, los patrones espaciales de las comunidades de diatomeas, macroinvertebrados y peces estuvieron marcados por un gradiente aguas arriba-aguas abajo. Sin embargo, también se identificaron respuestas biológicas significativas a variables ambientales dinámicas (es decir, hidrología, clima, uso del suelo). Esto sugiere que los procesos relacionados con el cambio global, como el cambio climático y la alteración hidrológica, podrían provocar importantes cambios en las comunidades fluviales estudiadas. En concreto, los climas más cálidos, la mayor proporción de terreno agrícola, los regímenes de caudal más estable y la geología calcárea favorecieron los rasgos más tolerantes (p.e., diatomeas móviles, macroinvertebrados de pequeño tamaño y peces no migratorios). Además, los rasgos de las diatomeas fueron más sensibles a las variables de LULC, los de los macroinvertebrados a la geología y al LULC, y los de los peces al clima, al LULC y a la hidrología.

Al investigar el papel de los factores espaciales (es decir, relacionados con la dispersión) y ambientales (es decir, el nicho) en la estructuración de las comunidades biológicas en el Capítulo 3, ambos factores mostraron una influencia significativa en el control de los patrones de variabilidad de las diatomeas y los peces. De hecho, los factores ambientales estructurados espacialmente fueron los que más contribuyeron a la variabilidad espacial de las comunidades biológicas analizadas, seguidos de las distancias espaciales y las variables puramente ambientales. Las comunidades de peces mostraron una mayor dependencia espacial que las diatomeas, lo que podría estar relacionado con su menor capacidad de dispersión y con el mayor tamaño de estos organismos. Estos factores no suelen tenerse en cuenta en los programas tradicionales de monitorización biológica o de conservación, que asumen que los patrones de las comunidades se estructuran basándose únicamente en las preferencias de las especies a lo largo de los gradientes ambientales. Sin embargo, la evaluación basada en esos criterios puede generar datos sesgados, ya que los nuevos colonizadores (p.e., peces u otras especies con capacidad de dispersión reducida) pueden ser incapaces de llegar a estos lugares con condiciones ambientales favorables, indicando, por tanto, un lugar potencialmente impactado cuando no lo está. En este contexto, hay que hacer hincapié en la necesidad de incorporar criterios de distancia espacial en el diseño del seguimiento de impactos o incluso en el establecimiento de condiciones de referencia, junto con el papel de las variables ambientales, ya que, de lo contrario, los esfuerzos de seguimiento y evaluación biótica podrían verse obstaculizados. Además, deberían tenerse en cuenta las capacidades de dispersión de las distintas comunidades biológicas utilizadas como indicadores biológicos. Por ejemplo, la conectividad de los hábitats podría monitorizarse mucho mejor a través las comunidades de peces, mientras que cambios en los nutrientes y hábitats podrían monitorizarse mejor a través de las diatomeas.

A escala nacional, las variables ambientales a nivel de cuenca (p.e., clima, LULC, hidrología, topografía y geología) contribuyeron en mayor medida a explicar la variabilidad de las comunidades de peces que la de los macroinvertebrados y las diatomeas en los ríos inalterados. Estos resultados indican que los patrones de las comunidades de peces podrían estar fuertemente influidos por factores ambientales a gran escala, lo que también se confirmó en el Capítulo 3. A su vez, estas variables ambientales a escala de cuenca tuvieron una menor capacidad predictiva para las comunidades de diatomeas, lo que sugiere que estas podrían estar respondiendo a otros factores locales (p.e., calidad del agua y características físico-químicas, condiciones del espacio ripario) que no han sido considerados en nuestros análisis. Por lo tanto, destacamos que la selección de bioindicadores, la escala del estudio y la escala de los factores ambientales podrían influir los resultados de la evaluación de la integridad ecológica del ecosistema fluvial.

Importancia de la alteración de las condiciones hidrológicas y fisicoquímicas

La importancia ecológica de diferentes atributos del régimen de caudales se puso de manifiesto en los Capítulos 4 y 5. La alteración de la magnitud de los caudales mensuales (caudales de invierno y verano, caudales de primavera) en ríos de montaña, que dieron lugar a una inversión de los patrones de caudales estacionales y la disminución en la amplitud de los picos de caudal fueron las principales alteraciones hidrológicas asociadas a las presas con fines agrícolas. Las presas hidroeléctricas presentaron un elevado número de pulsos de caudal de corta duración, que se tradujo en una variación de la tasa de cambio caudal respecto a las condiciones de caudal natural. En cuanto a las variables físico-químicas, las presas de uso agrícola redujeron considerablemente las concentraciones de nutrientes y SST aguas abajo, mientras que las presas hidroeléctricas presentaron temperaturas del agua significativamente más altas en comparación con los controles.

Los parámetros físico-químicos desempeñaron un papel clave para explicar la variabilidad de las comunidades biológicas y mostraron una respuesta clara a la presencia de presas aguas arriba, tal como se demostró en el Capítulo 5. Como se ha mencionado anteriormente, las bases de datos a escala nacional utilizadas en los Capítulos 2 y 3 no contaban con este tipo de información, lo que posiblemente impidió explicar un mayor porcentaje de la variación de la distribución de las comunidades, afectando especialmente a las comunidades de diatomeas, al tratarse organismos más dependientes de las características locales (nicho). Considerando estos resultados, se hace patente la necesidad de incorporar variables físico-químicas para evaluar correctamente los patrones de distribución y la respuesta de las comunidades fluviales.

Los 35 índices hidrológicos calculados a partir de los datos de caudal diario demostraron ser elementos clave para entender completamente y de un amanera estructurada la variación del régimen de caudales es estado natural y la alteración hidrológica causada por las presas. Junto con los parámetros físico-químicos, los índices hidrológicos permitieron establecer relaciones clave con las comunidades biológicas y sus rasgos. Estos resultados refuerzan la necesidad de incorporar índices hidrológicos (o métricas hidrológicas con relevancia para la ecología) en los programas de monitorización y conservación, así como de establecer lugares de estudio de las comunidades biológicas que estén emparejados con estaciones de aforo.

Las características de la presas, incluyendo su capacidad de regulación o sus normas y esquemas de funcionamiento, demostraron ser factores cruciales que determinan la respuesta de las comunidades biológicas, como se demuestra en los capítulos 4 y 5. Esta conclusión sugiere que la elaboración de estrategias de

mitigación del impacto de las presas y el establecimiento de caudales ecológicos deberían considerar las características específicas de cada presa.

Contribuciones a los programas de monitorización biológica y conservación

Los hallazgos de esta tesis plantean puntos críticos que podrían contribuir a la mejora de las actuales estrategias de monitorización y conservación de los ecosistemas fluviales. La consideración de organismos con distinta naturaleza ha sido fundamental para profundizar en el conocimiento de cómo diferentes factores ambientales, modificados en respuesta a la presencia de una presa, pueden influir en tres tipos de comunidades biológicas. Las diatomeas, los macroinvertebrados y los peces, es decir, los organismos utilizados habitualmente como indicadores biológicos en los programas de seguimiento, mostraron respuestas variables a distintos factores de estrés, predictores ambientales y espaciales y escalas de estudio. Estos resultados proporcionan información sobre cuales deberían ser los bioindicadores más adecuados para realizar un seguimiento de cada factor de estrés y los cambios esperados en las comunidades, lo que puede contribuir al desarrollo de relaciones caudal-respuesta ecológica y a programas de monitorización más eficaces.

El uso de rasgos a lo largo de los cuatro capítulos puso de relieve sus beneficios frente al uso exclusivo de la taxonomía. Por ejemplo, la mayoría de los cambios en las comunidades fluviales identificados en esta tesis pueden asociarse a la sensibilidad de los organismos a factores de estrés o gradientes ambientales, por ejemplo, los rasgos asociados a una mayor resistencia, como los ciclos vitales cortos y los tamaños corporales pequeños, predominaron aguas abajo de las presas. Las respuestas basadas en rasgos permitieron establecer una comprensión mecanicista de cómo se estructuran las comunidades a través de los gradientes ambientales, los efectos de las presas u otros factores de estrés, facilitando la predicción de las respuestas biológicas a los impactos humanos. Además, el uso de rasgo permitió unificar el pool de especies de las diferentes ecorregiones, un punto especialmente importante para los peces, ya que este grupo está sujeto a importantes restricciones biogeográficas, que se traduce en una importante diversidad específica entre cuencas.

El uso de clases hidrológicas en régimen natural en el Capítulo 5 resultó acertado en comparación con otros capítulos, ya que redujo el ruido ambiental e hidrológico, permitiendo centrarse en los impactos relacionados con las presas. En el Capítulo 4, aunque la selección de emplazamientos se limitó a los arroyos de montaña, el ruido generado por la variabilidad ambiental e hidrológica dificultó la interpretación de los efectos relacionados con las presas sobre las comunidades biológicas. Además, la

clasificación hidrológica, que es un paso esencial en ELOHA (Poff et al., 2010), nos permitiría exportar las relaciones caudal-respuesta ecológica halladas en la zona de estudio del Capítulo 5 para proporcionar estimaciones de caudal ecológico a otros ríos pertenecientes a las mismas clases fluviales sin necesidad de un trabajo tan exhaustivo exhaustivo.

En resumen, los resultados y enfoques explorados en esta tesis doctoral pueden contribuir al desarrollo de recomendaciones más eficaces en el marco del diseño de regímenes de caudales ecológicos y de otros esfuerzos de mitigación de los impactos de las presas. Este es el caso del uso de rasgos y de clasificación hidrológica, estrategias adoptadas en este estudio para tratar las grandes escalas espaciales y que pueden contribuir eficazmente al desarrollo de recomendaciones regionales de caudales ecológicos. Además, el uso de diatomeas, macroinvertebrados y peces y sus rasgos puso de relieve las diferencias entre estos organismos en términos de respuesta y sensibilidad a factores de estrés específicos, la dispersión y escala espacial, que son esenciales a la hora de diseñar programas de monitorización y establecer relaciones caudal-respuesta ecológica para su aplicación a las políticas de gestión del agua.

Futuras líneas de investigación y recomendaciones

Desde hace varias décadas la importancia de los regímenes de caudales para la biodiversidad y los ecosistemas fluviales está cada vez más reconocida en, por lo que la implantación de caudales ecológicos se ha convertido en una tarea urgente en la gestión del agua. Sin embargo, como se ha señalado a lo largo de esta tesis, aún es necesario seguir investigando y profundizando en varios aspectos relacionados con la monitorización y la conservación de los ecosistemas fluviales. Los puntos que se enumeran a continuación destacan algunas de estas lagunas de conocimiento, indicando algunas recomendaciones y oportunidades para el desarrollo de nuevas líneas de investigación:

- Las relaciones entre predictores ambientales (p.e., gradientes ambientales, índices hidrológicos, variables fisicoquímicas) y respuestas biológicas identificadas en este estudio se basaron en correlaciones. Por lo tanto, sería esencial realizar experimentos en colaboración con los operadores de las presas para validar las relaciones caudal-respuesta ecológica, que serían una herramienta esencial para definir e implementar regímenes de caudales ecológicos adecuados (p.e., dentro del marco ELOHA) y otras medidas de mitigación del impacto de las presas.

- Sería interesante explorar las interacciones entre los tres grupos bióticos considerados en esta tesis para comprender mejor la dinámica del ecosistema fluvial y la red trófica, así como para determinar los efectos en cascada en respuesta a las presas o a otros factores de estrés (p.e., asociar los cambios en las comunidades de diatomeas a los cambios en las comunidades de macroinvertebrados y peces desde una perspectiva de red trófica). Además, una evaluación más profunda de la congruencia entre taxones de los grupos bióticos estudiados sería beneficiosa para los programas de monitorización.

- Los métodos actuales de monitorización biológica asumen que las comunidades están controladas únicamente por las condiciones abióticas locales (es decir, enfoque de nicho). La incorporación de procesos espaciales, relacionados con la dispersión, la conectividad y los conceptos de metacomunidad y metaecosistema, especialmente en ecosistemas muy dinámicos (p.e., ríos intermitentes; Cid et al., 2020), mejoraría la precisión de los enfoques actuales de monitorización para detectar los efectos de los factores del cambio global en los ríos.

- La falta de índices bióticos sensibles a al efecto de las presas subraya la necesidad de generar métricas bióticas específicamente diseñadas para detectar la alteración hidrológica y otros impactos relacionados con las mismas. En este contexto, el desarrollo y calibración de índices bióticos basados en rasgos biológicos sensibles, como los identificados en este estudio, contribuiría a una mejora en la evaluación de los impactos de las presas.

- Deberían explorarse metodologías adicionales basadas en las relaciones entre regímenes de caudal, variables ambientales y comunidades biológicas obtenidas en este estudio. Por ejemplo., la construcción de modelos predictivos que combinen los diferentes grupos taxonómicos, la variación temporal de los muestreos biológicos y otros parámetros ambientales críticos (p.e., clases hidrológicas).

- Como se ha visto en los capítulos 2 a 4, la información biológica recogida para la evaluación de la calidad del agua por las confederaciones hidrográficas y las agencias del agua a escala nacional ha demostrado ser útil más allá de su propósito original. Así pues, los datos recogidos para evaluar el estado ecológico de las masas de agua en toda Europa en el contexto de la DMA deberían catalogarse en una base de datos unificada y divulgarse con fines de investigación. Aunque los problemas relacionados con la distribución espacial y temporal de estos datos y su resolución taxonómica podrían suponer una limitación para su uso en la investigación, los estudios sobre biodiversidad, patrones de distribución y el desarrollo de relaciones preliminares entre caudal

y la respuesta ecológica para la definición de caudales ecológicos, podrían potenciarse con el uso de conjuntos de datos fluviales a gran escala.

- El fomento de colaboraciones entre operadores de presas, gestores de recursos hídricos e investigadores podría ser muy beneficiosa para producir registros a largo plazo de los efectos de las presas en los ecosistemas fluviales y abordar las lagunas de conocimiento críticas. En este contexto., en el río Spöl (Suiza) se promovieron y monitorizaron treinta eventos de crecida experimentales entre 2000 y 2016, lo que contribuyó profundamente a comprender la dinámica del ecosistema y las respuestas de la biota a largo plazo (perifiton, macroinvertebrados y peces) en los ríos alpinos (Robinson et al., 2018). Otros estudios también han informado de que estos experimentos a gran escala, en cooperación con los operadores de presas, son cruciales para lograr resultados óptimos que permitan asesorar sobre las mejores estrategias gestión y políticas de operación de presas (Olden et al., 2014).

Chapter 1

Introduction and background to the research

1.1 Earth's surface freshwaters

Freshwater accounts for only 0.01% of all available water on Earth (Lehner and Döll, 2004). Despite that, their ecosystems harbour a disproportionately high fraction of the earth´s biodiversity: around 10% of all known species and a third of all vertebrates (Strayer and Dudgeon, 2010). Freshwaters also provide a range of essential ecosystem services, such as water for drinking and irrigation, food (e.g. fish), cultural values (e.g. recreation, ecotourism, religiosity), and support other services like soil formation, nutrient cycling, and habitat provisioning (Durance et al., 2016; Petsch et al., 2022).

The management of these ecosystems, however, has often prioritised a limited range of services for macroeconomic gain to the detriment of aquatic habitats, their flora, fauna, and the diverse benefits they offer to societies (Tickner et al., 2020b). Around 65% of the aquatic habitats supported by freshwater are subjected to multiple stressors emerging mainly from anthropogenic activities (WWAP, 2019), such as the changing climates, flow regulation caused by dams, water pollution, and invasive species (Dudgeon, 2019; Reid et al., 2019; Vörösmarty et al., 2010).

1.1.1 The current state of freshwater systems

The pervasive geological, sociological, anthropological, and ecological changes in recent Earth's history, encompassing the "Anthropocene", have brought overwhelming impacts on ecosystems, being particularly relevant to freshwaters (Heino et al., 2021; Waters et al., 2016). Consequently, the current scale of biodiversity loss in freshwater ecosystems is alarming. The Freshwater Living Planet Index showed an important decrease in freshwater populations (equivalent to 4% per year since 1970), especially in amphibians, reptiles, and fishes across all regions (Baker et al., 2020). Wetlands have lost approximately 35% of their area since the 1970s, which corresponds to three times the rate of forest loss; as a consequence, 81% of wetlands species have shown declines in population size (Gardner and Finlayson, 2018).

Overexploitation, water pollution, flow alteration, and invasion of exotic species are among the major causes of freshwater biodiversity loss (Dudgeon, 2019; Dudgeon et al., 2006; Reid et al., 2019). Nevertheless, the lower investments targeting freshwater ecosystem conservation in comparison to their terrestrial counterparts contrast with their degree of jeopardy (Abell et al., 2011). Freshwater ecosystems are in urgent need of effective management strategies and actions to halt biodiversity loss. Their degree of effectiveness will depend on the ability to preserve the natural habitat configuration along with population dynamics, as well as a deep knowledge on the main drivers governing biota distribution patterns (Allan and Flecker, 1993; He et al., 2018; Olden et al., 2010; Tickner et al., 2020b).

Since the development of global change programmes in the 1980s and the Dublin Conference on Water in 1992, water-related concerns have been discussed in international sustainability agendas. The United Nations Sustainable Development Goals (SDGs), implemented in 2015, included targets to protect and restore water-related ecosystems, and the Convention on Biological Diversity (CBD) adopted the Strategic Plan for Biodiversity 2011-2020 and the Aichi 2020 Targets. However, in comparison to terrestrial, coastal, and marine areas, inland waters are overlooked in these global conservation and restoration actions, and only the most immediate freshwater ecosystem services are prioritised (Gonçalves and Hermoso, 2022). Thus, greater attention to freshwaters is requested by scientists in the Post-2020 Global Biodiversity Framework (GBF), with conservation and restoration actions prioritising these ecosystems (GEO BON and FWBON, 2022).

Tickner et al. (2020b) proposed a recovery strategy to reverse the decline in freshwater biodiversity and guide future global biodiversity frameworks and actions. This emergency plan (Figure 1.1) is structured around the major causes of freshwater biodiversity loss identified by Dudgeon et al. (2006) and prioritises actions such as the

implementation of environmental flows, water quality improvement, the protection and restoration of critical habitats, and freshwater connectivity.

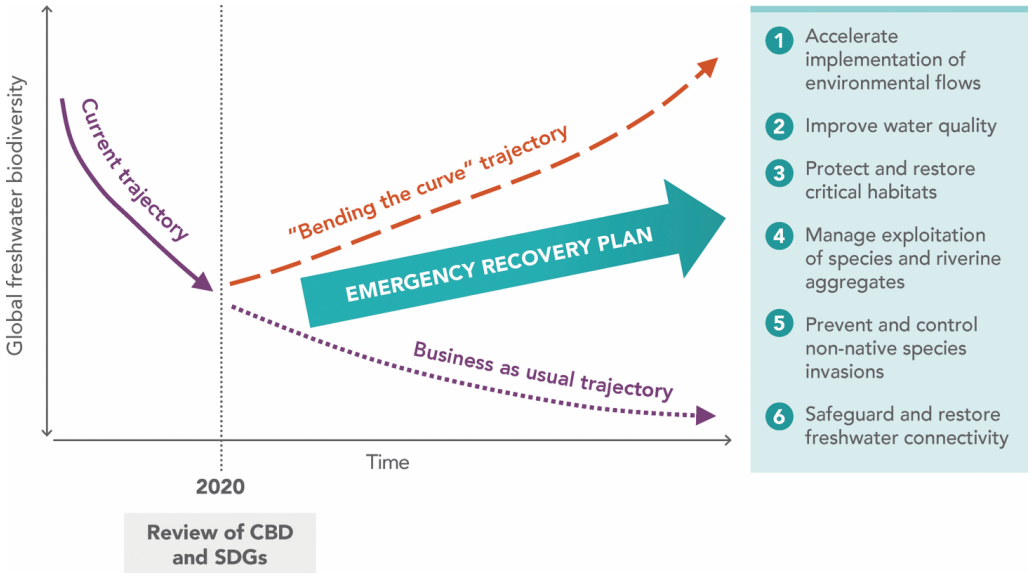


Figure 1.1 Emergency recovery plan to bend the curve of freshwater biodiversity loss, proposed by Tickner et al. (2020b).

1.1.2 The current state of freshwaters in Europe

The implementation of the European Water Framework Directive (WFD; European Commission, 2000) in 2000 was a milestone in the water policy of the European Union Member States. As its primary goal, the WFD established a framework for the restoration of aquatic systems to achieve good ecological status for all European rivers, lakes, and wetlands.

The WFD shifted the water management paradigm from an anthropocentric to an ecocentric perspective, and water was then seen as an ecosystem holder instead of a resource to be exploited by societies (European Commission, 2000; Santos et al., 2021). Water bodies' ecological status started to be evaluated biologically rather than only by their physico-chemical characteristics, shifting management objectives from pollution control to ecosystem integrity as a whole (Hering et al., 2010).

After the first management cycle (2010-2015), which was initially set as the deadline for achieving the WFD main objectives, the river basin management plans (RBMPs) indicated that around 60% of surface waters failed to achieve good ecological status. In general, rivers and transitional waters scored lower than coastal waters and lakes (European Environment Agency, 2018a), and most of the poor-condition water bodies were located in central Europe. The Member States have then the chance to achieve a good ecological state by 2021 or, at the latest, by 2027 (Ramos et al.,

2018). In contrast, a high proportion of water bodies in the northern Scandinavian, Scotland, Estonia, Romania, Slovakia, and the Mediterranean region was reported to be in good ecological status (Figure 1.2).

In comparison to the 2009 RBMP reports, around 20% of the surface water bodies (i.e. 16,000 water bodies) showed some progress towards a good ecological status in the 2015 report (European Commission, 2019a). In many cases, improvements in individual quality elements (i.e. physico-chemical, hydromorphological, and biological) were detected; however, they did not reflect an improvement of the overall ecological status due to the conservative "one-out-all-out" principle (i.e. the lowest score of any quality element determines the overall ecological status of the water body; European Commission, 2019a; Santos et al., 2021).

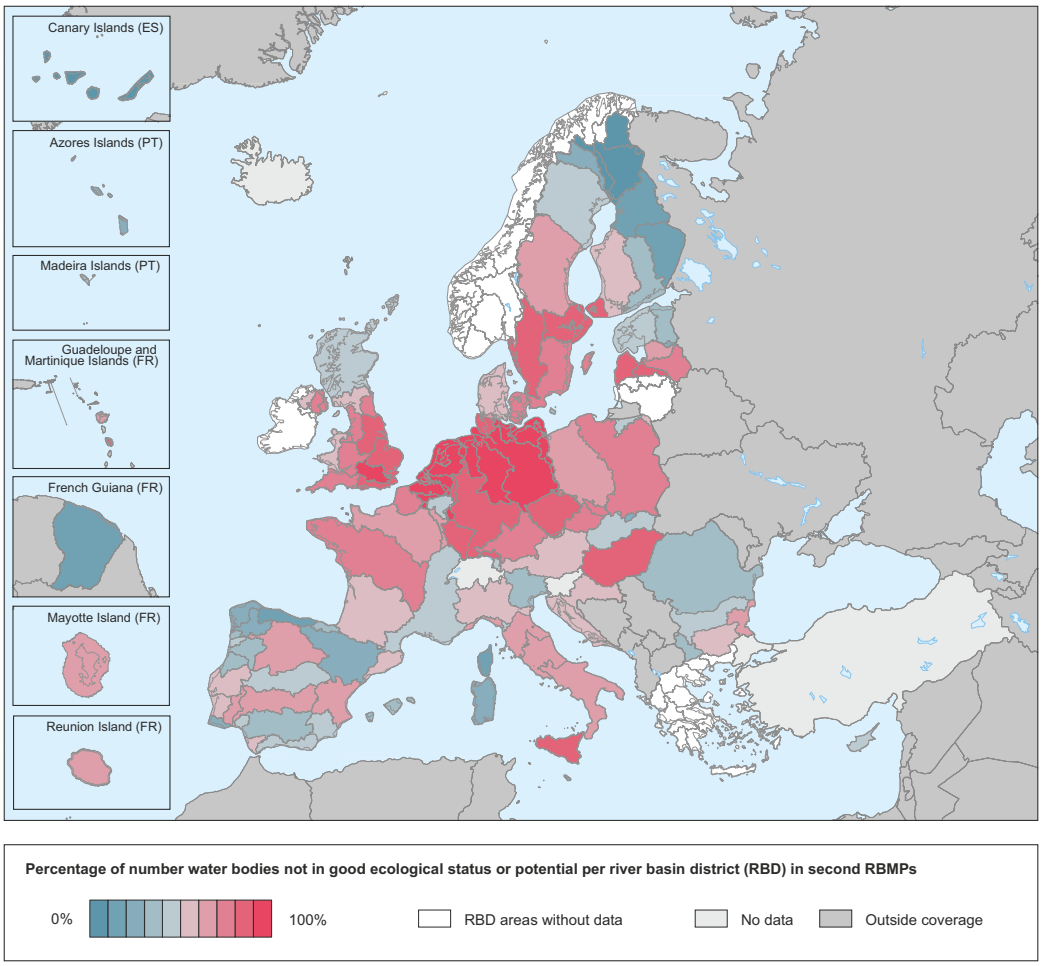


Figure 1.2 Percentage of water bodies in the European Member States that did not meet good ecological status in the second RBMPs (European Commission, 2019a).

Although the Member States did not yet succeed in achieving the Directive's main goal, the WFD promoted a deeper knowledge of European freshwater resources. The number of water bodies assessed progressively increased, as well as the general confidence in the status assessment (European Environment Agency, 2018a).

Moreover, the Directive promoted the development and use of comparable ecological assessment methodologies across Europe, considerably improving the effectiveness of monitoring programmes (Birk et al., 2012; Carvalho et al., 2019).

1.1.3 Global water uses

Global water consumption has grown sixfold over the past century, with an annual increment of 1.8% (Boretti and Rosa, 2019; Wada et al., 2016). These increases can be attributed to a combination of population growth, economic development, and shifts in consumption patterns (Flörke et al., 2013; United Nations, 2021).

The agriculture sector is the main water user, accounting for 69% of the global consumption and reaching up to 95% in some developing countries (FAO, 2011). Industry, energy, and power generation account for 19% of water consumption worldwide, while the remaining 12% corresponds to domestic water use (Figure 1.3).

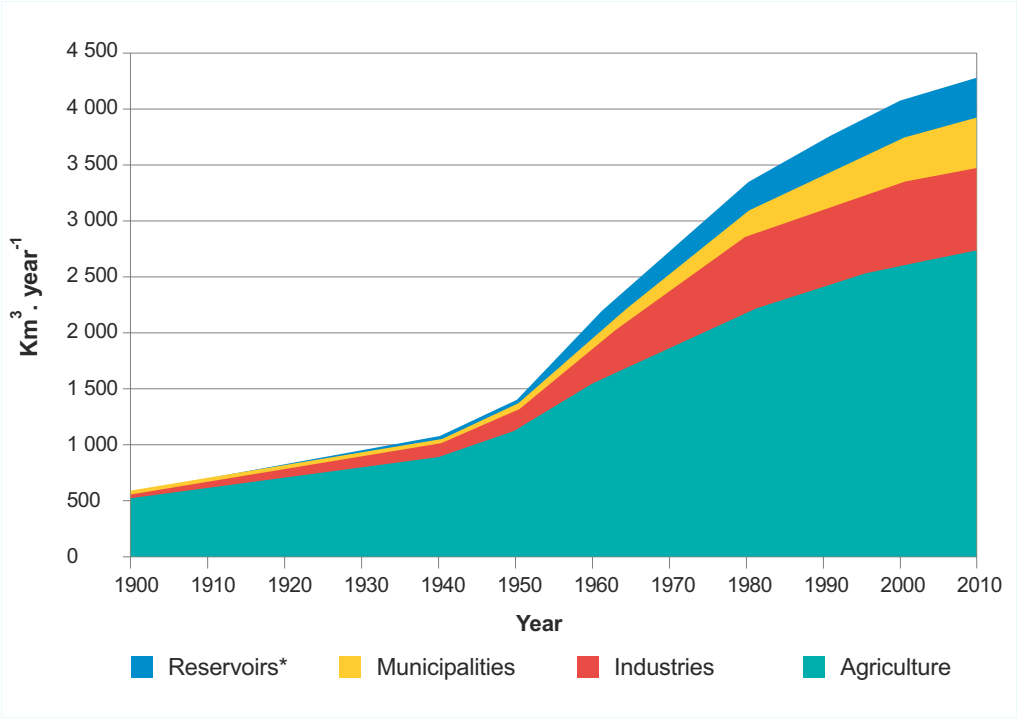


Figure 1.3 Global water consumption between 1900 and 2010 (AQUASTAT, 2010; United Nations, 2021). *Reservoirs refer to the evaporation from artificial lakes.

The global demands are expected to grow significantly over the next decades, particularly the domestic and industrial ones in emerging economies, middle-, and low-income countries (Ritchie and Roser, 2017; United Nations, 2021), therefore improving water availability (e.g. water desalination and reservoir construction) and reducing water consumption (e.g. efficient irrigation methods, water recycling; He et al., 2021) is essential.

1.1.3.1 Water uses in Europe

In Europe, the major water user in 2017 was agriculture (58%), followed by cooling water for energy production (18%), manufacturing, mining, and construction industries (14%), and households (10%; European Environment Agency, 2021). However, these trends varied across the Member States: in northern countries, industry and electricity production were the major water consumer (67% in 2017), while in southern countries, agriculture was the major consumer (80% in 2017). In general, surface waters contributed to 76% of this total annual water abstraction, and groundwater to around 24% (Figure 1.4a; European Environment Agency, 2021, 2022).

Annual water uses in EU Member States declined by 15% between 2000 and 2019 (13,000 million m^3; European Environment Agency, 2022). The water use efficiency increased in water-dependent sectors (e.g. agriculture, energy production, domestic water supply, and tourism) and the production grew by 20% of the net value added (European Environment Agency, 2021). This decrease in water consumption is also related to the replacement of combustion plants that require water for cooling by renewable energy sources, as part of the new European Green Deal (European Commission, 2019b).

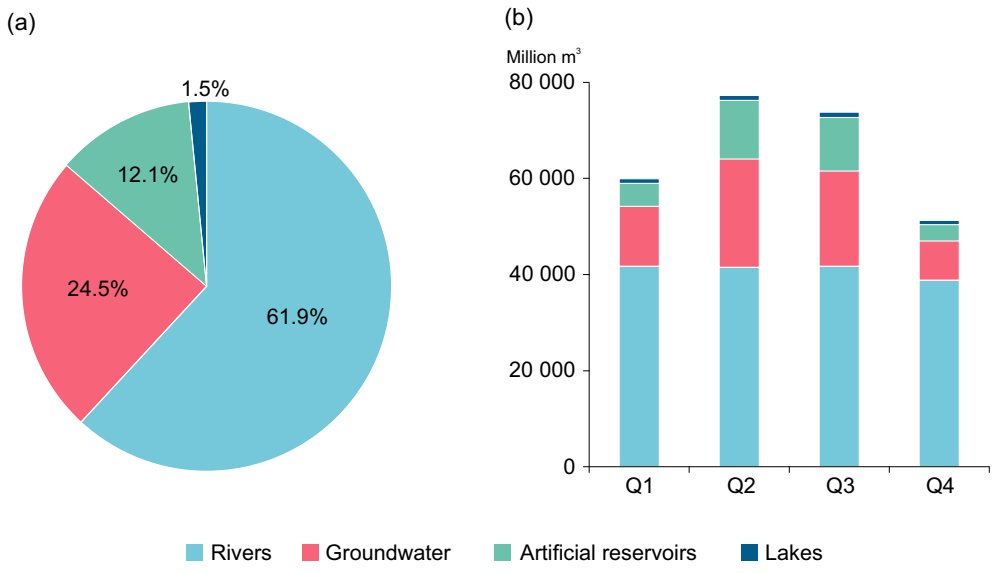


Figure 1.4 (a) Annual and (b) seasonal freshwater abstraction by source in Europe (EEA-38 and the UK). Q1: January, February, and March, Q2: April, May, and June, Q3: July, August, and September, Q4: October, November, and December. Source: European Environment Agency (2021).

However, the relative contribution of groundwater abstraction grew over the same period. This increase (from 19% in 2000 to 23% in 2019) was motivated by the increasing water stress in surface water bodies exacerbated by climate change,

especially in southern Europe during spring and summer (Figure 1.4b; European Environment Agency, 2021).

1.2 Dams and their ecological impacts

River impoundments, dams, diversion weirs, run-of-river abstraction, and other water exploitation infrastructures have been built and used to meet the diverse water demands of our societies (Tharme, 2003). Their primary function is to retain water in periods of high flow and release it gradually during lower flow periods, when it is most needed (Baxter, 1977). In the case of hydropower dams, as the energy demand varies daily, the water can be retained during the night or during the high flow seasons and released gradually or abruptly, depending on the electricity needs (e.g. base or peak demands; Egré and Milewski, 2002).

The number of large dams worldwide (≥15 m height, impounding more than 3 hm^3) climbed from 5,000 in 1950 to over 58,000 dams in 2020 (ICOLD, 2021), and nowadays only 37% of the large rivers worldwide remain free-flowing (Grill et al., 2019). This rapid growth in dam construction can be attributed not only to the high water and energy demands but also to the need to reduce greenhouse gas emissions as part of sustainable development initiatives (Zarfl et al., 2014).

These hydraulic structures produce sound impacts on the watershed and riverine ecosystems in where they are built. Among the numerous consequences, which vary with dams' size, location, and operational scheme (Poff and Hart, 2002), they can alter the flow dynamics (Poff et al., 2007), the downstream flux of water and sediment (Magilligan and Nislow, 2005), the biogeochemical cycles (Maavara et al., 2020), water temperatures (Olden and Naiman, 2010), and interrupt river lateral and longitudinal connectivity (Barbarossa et al., 2020; Grill et al., 2015).

1.2.1 Dam purposes

Water supply dams release water according to urban, agricultural, and industrial water demands. Depending on their size, these dams can completely rearrange seasonal patterns of flow regime, as they usually capture wet-season flows to release them during the dry season to support irrigated agriculture (Richter and Thomas, 2007; Schmutz and Moog, 2018). In most cases, the water impounded is diverted directly from the reservoir, reducing significantly the amount of water downstream (Richter and Thomas, 2007).

Large hydropower dams usually have a considerable storage capacity to operate following the energy demands and may cause abrupt changes in the downstream hydrological patterns (Poff and Hart, 2002). This intermittent power generation results in frequent discharge pulses of water, which are commonly referred to as "hydropeaking" (Greimel et al., 2018). These artificial discharge variations cause extreme and sudden fluctuations in the river flow, which cascade down on important geomorphological re-adjustments on river channel morphology, water depth and velocity patterns, and composition of suspended material, among other numerous effects (Moog, 1993; Schülting et al., 2019). In contrast, hydropower dams operating as "run-of-river" have little or no storage capacity (Richter and Thomas, 2007). The absence of a reservoir reduces considerably the ecological and social impacts; however, their energy production may vary significantly during the year, as it depends on the natural river flow (Egré and Milewski, 2002).

Flood control dams capture a portion of the floodwaters to subsequently release it at a lower discharge rate (Richter and Thomas, 2007). Their main purpose is to delay the process in which precipitation is converted into runoff and reaches the river, also contributing to desynchronise tributary peak flows (Green et al., 2000). Furthermore, impoundments can be constructed to create a new body of standing water for social and economic purposes, such as heat dissipation in a thermoelectric plant, fishing, tourism, recreation, and religious values (Richter and Thomas, 2007).

1.2.2 Main impacts caused by dams

The process of river damming creates a new ecosystem differing from streams and lakes (Baxter, 1977). The flow regime and its dynamism are the fundamental factors defining the riverine physical structure and ecological processes (Poff et al., 1997), thus, alterations in the flow regime by dams cause immediate changes in other ecosystem components, such as the energy and matter fluxes, connectivity, and biota distribution (Figure 1.5).

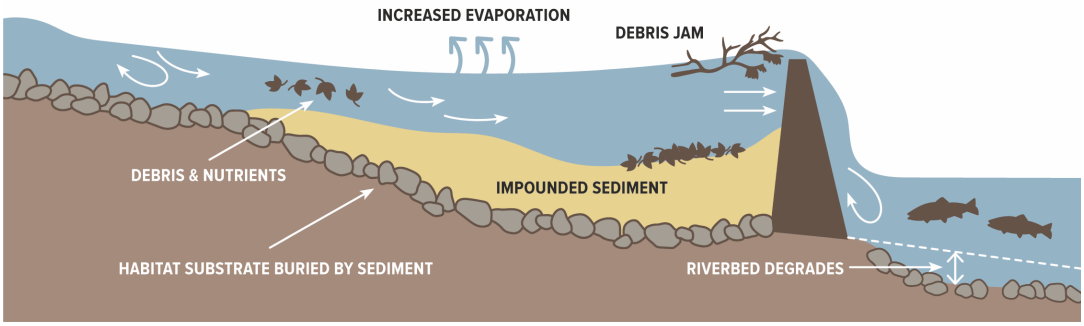


Figure 1.5 Dam-related impacts in rivers. Adapted from The Boardman River Dams Project (http://theboardman.org/dam-project).

1.2.2.1 Flow regime alteration

The natural flow paradigm (sensu Poff et al., 1997) predicts that the flow regime, which can be described by five attributes (i.e. magnitude of discharge, frequency, duration, timing, and rate of change), sustains the full native biodiversity and integrity of aquatic ecosystems (Poff et al., 1997; Richter et al., 1996, 1997). However, dams tend to homogenise the flow variability primarily through changes in the magnitude, frequency, and timing of high and low flows (Magilligan and Nislow, 2005; Poff et al., 2007). For example, the natural high flows, besides their importance in allowing species to colonise downstream zones (Ward and Stanford, 1995), contribute to exporting sediments and organic resources (Schmutz and Moog, 2018). Therefore, alteration of the flow attributes may lead to a wide range of responses in ecological and geomorphological variables (Lloyd et al., 2004; Poff and Zimmerman, 2010).

The linkages between flow and riverine biota can be summarised with the key flow-biota principles proposed by Bunn and Arthington (2002), shown in Figure 1.6. The river morphology is largely determined by the interaction between the flow regime and local geology; simultaneously, the river shape and size set the biota distribution and diversity (Principle 1), and the flow timing determines the species' life cycles (Principle 2). The high flow events connect habitats, expand the river network, and allow species to access new areas (Principle 3). Thus, since the first three principles set ideal conditions for native species, modified flow conditions can lead to biodiversity loss and facilitate invasions of exotic species (Principle 4).

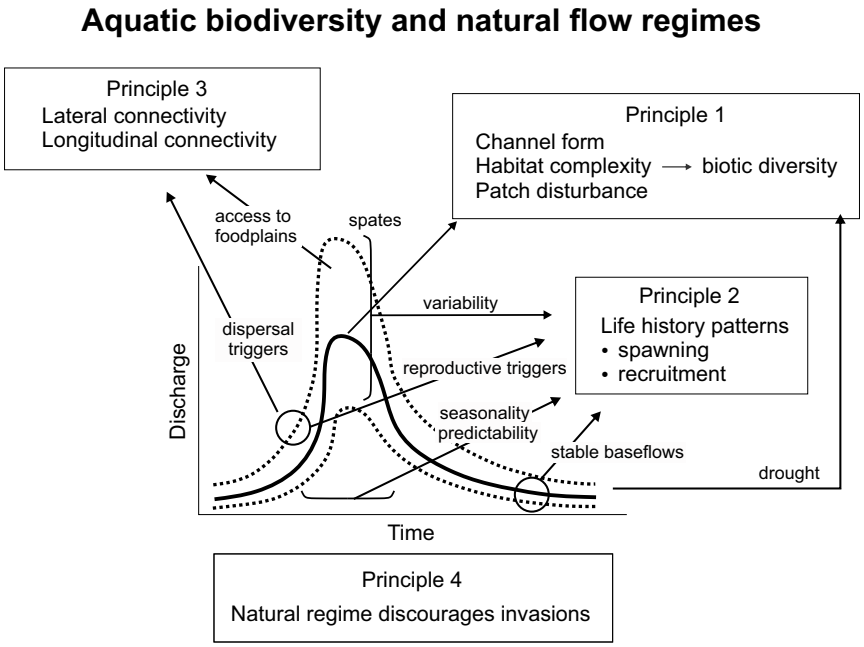


Figure 1.6 Key flow-biota principles highlighting the importance of river flow regime and its components. Source: Bunn and Arthington (2002).

1.2.2.2 Sediment and nutrient fluxes

Sediment trapping is a well-known impact of dams and reservoirs (Schmutz and Moog, 2018) and is aggravated by the accelerated construction of dams and land-use changes worldwide. Recently, Dethier et al. (2022) reported a reconfiguration of the global patterns in sediment fluxes to the oceans. In the global north, sediment trapping by dams has decreased the sediment flux to the sea to nearly half of the pre-dam conditions, whereas in the global south, the suspended sediment concentration in rivers was 41% greater than in the 1980s, which can be attributed to the intensive land-use change and erosion.

As dams interrupt the continuity of sediment transport through the river system, the sediments accumulate within the reservoir, resulting in a loss of its storage capacity, clogging outlet structures, and decreasing the efficiency of turbines and other hydraulic equipment (Schleiss et al., 2016). At the same time, the downstream reaches are deprived of essential sediments that sustain riverine ecosystems, the bed material becomes coarser, and critical habitats are modified, e.g. salmon and trout spawning beds (Kondolf, 1997; Kondolf et al., 2014).

Measures to mitigate reservoir sedimentation involve actions at different scales. Upstream of the reservoir, soil conservation techniques and changes in crop practices, increase of the riparian vegetation, and slope stabilisation may reduce the sediment production at the catchment level (Kondolf et al., 2014). Bypass tunnels, water jets to avoid fine sediment deposition, mechanical excavation, dredging, pressure flushing, and increase of outlet level are other measures applicable in reservoirs and dams (Kondolf et al., 2014; Schleiss et al., 2016).

Similarly, the nutrient (e.g. carbon, phosphorous, nitrogen, silicon) fluxes along the river network are blocked by dams, altering the nutrient ratios with their elimination from the water column via sediments burial or gaseous releases into the atmosphere (Maavara et al., 2020). It leads to pervasive effects in the riverine ecosystems, altering the river metabolism (González et al., 2013; von Schiller et al., 2016), the structure of food webs at all levels (Glibert, 2012), and reducing macroinvertebrate density (Kennedy and Turner, 2011) and salmon populations (Moore et al., 2011).

1.2.2.3 Water temperature

Water temperature is a key physical variable in riverine ecosystems, influencing the stream metabolism, biodiversity distribution, life history, and growth rates of the aquatic biota (Buisson et al., 2008), e.g. macroinvertebrate growth and egg

development (Elliott, 1978; Vannote et al., 1980) and salmonid spawning (Flodmark et al., 2004; Isaak et al., 2010).

Dams and reservoirs alter the riverine thermal regimes in numerous ways (Niemeyer et al., 2018). Large dams (≥15 m height) tend to be thermally stratified due to the water density varying with temperature. In summer, a relatively warm surface layer (epilimnion) separates from the cooler, denser water (hypolimnion; Petts, 1986). Thus, in bottom-release reservoirs, the hypolimnetic water releases lower the downstream water temperature (Hester and Doyle, 2011). In contrast, in small dams (e.g. run-of-river hydropower dams), the temperature is expected to increase downstream due to the lower velocity, increased surface of the impoundment, and solar radiation (Chandesris et al., 2019).

Dam-induced changes in the temperature regime have a strong influence on river biota. Previous studies showed that the temperature constancy downstream of dams was a critical factor for macroinvertebrate persistence and taxonomic richness (Rader et al., 2008), and increased temperatures were linked to a reduction in cold-water fish populations (Hayes et al., 2006; Lessard and Hayes, 2003).

Although thermal impacts are seldom acknowledged in strategies to mitigate dam impacts (Olden and Naiman, 2010), dams with multiple release points spanning the entire depth of the reservoir allow water to be released from different depths at certain periods of the year to mimic the natural temperature variation (Rader et al., 2008).

1.2.2.4 River network connectivity

Dams and weirs function as an artificial barrier to the longitudinal movement, which is amplified by the river's dendritic spatial configuration (Crook et al., 2015). In addition to the aforementioned impacts, dams also disrupt the organisms' natural patterns of dispersal, altering the structure, composition, and dynamics of many local populations (Rolls et al., 2013). It affects directly fish communities, which perform a diversity of migratory movements along the river network and are entirely confined to the hydrological connections in the system (Agostinho et al., 2008; Landeiro et al., 2011). Previous studies have reported the accumulation of juveniles directly below dams as their migration paths are blocked, as well as a reduction in fish taxonomic richness (Gehrke et al., 2002). González-Ferreras et al. (2022) also found that the presence of barriers caused a strong decrease in genetic diversity and population size of brown trout in isolated reaches. Flying insects or diatoms, in contrast, may be less affected by habitat fragmentation as they are able to reach other branches of the river network or even neighbour catchments (Liu et al., 2013).

Some structural or operational changes, including the installation of fish passages and periodical flushing flow releases, can reduce connectivity issues (Bravo-Córdoba et al., 2022). Fish ladders consist of a constantly open baffled path for fish, such as salmonids and other anadromous species, to reach upstream spawning and rearing habitats; in the case of large dams, fish can also be moved upstream by fish lifts (Schilt, 2007). However, these structures may delay or prevent the migration process as fish may have problems locating entrances or the passage size, slope, or discharge may not be ideal (Jonsson and Jonsson, 2011). For instance, only 47% of salmons succeed in reaching upstream through a fish lift in the River Garonne, France, as they were not sufficiently attracted to reach the entrance (Croze et al., 2008), and salmon migration was substantially delayed due to low water discharge in a fish passage in Norway (Thorstad et al., 2003).

Dam removal is also being increasingly considered for river restoration (Magilligan et al., 2016; O'Connor et al., 2015), although it is usually motivated by socioeconomic reasons, e.g. economically inefficient power production, unsafe structures, or abandoned dams (Bednarek, 2001). The removal of barriers provides major environmental advantages by re-establishing the upstream-downstream fluxes of sediment, nutrients, and organisms (Magilligan et al., 2016). However, it can also have negative implications, such as the passage of non-native species (e.g. control of the sea lamprey populations in the Great Lakes in North America; Miehls et al., 2020) and the release of contaminated sediments (Bednarek, 2001).

1.3 Mitigating the impacts of dams: Environmental flow methods

Considering the impacts of dams and reservoirs in freshwater ecosystems and the challenges faced by societies related to the water-energy-food (WEF) nexus, different strategies have emerged to reduce the many effects that these water infrastructures produce on river ecosystem health.

The first initiatives toward water bodies' protection emerged in the late 1940s in the United States in snowmelt streams to protect cold-water fisheries (Poff et al., 2017). Other conservation efforts in the United States and western Europe at this time were limited to setting minimum flows to mitigate water quality impairment (Poff et al., 2007). In the 1970s, significant progress in the conservation of rivers came with the development of new legislation (the US Clean Water Act; 92nd U.S. Congress, 1972) and the peak of dam construction. From the 1990s onward,

environmental water concepts and practices showed a strong expansion globally (Poff et al., 2017).

The Brisbane Declaration in 2007 and 2018a was a fundamental initiative to promote the global implementation of environmental flows. The term environmental flows (or "e-flows") was then defined as "the quantity, timing, and quality of freshwater flows and levels necessary to sustain aquatic ecosystems which, in turn, support human cultures, economies, sustainable livelihoods, and well-being". In this Declaration, scientists, river conservationists, and water managers set up recommendations to support environmental flow implementation, which are part of a critical step for achieving water-related SDGs (Tickner et al., 2020a). In this context, the recognition of certain flow requirements to sustain the structure and function of the entire riverine ecosystem and local livelihoods led to the development of holistic environmental flow methods (Poff et al., 2017; Zeiringer et al., 2018), for example, the Ecological Limits of Hydrological Alteration (ELOHA, Figure 1.7; Poff et al., 2010). This widely accepted framework is based on hydrological and biological monitoring, the regional grouping of rivers, the development of transferable relationships between flow alteration and ecological responses, adaptive management strategies, and societal needs and goals.

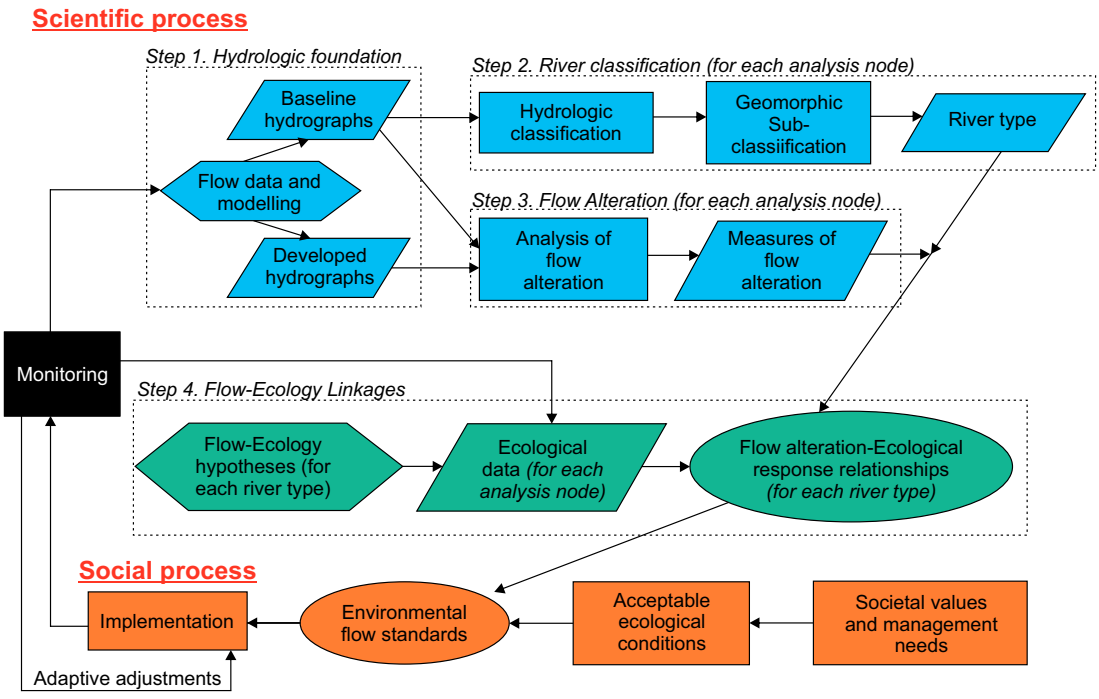


Figure 1.7 Summary of the ELOHA, a holistic framework in which hydrological, ecological, and social aspects are taken into account to assess hydrological alteration effects. Source: Poff et al. (2010).

1.3.1 Challenges in the environmental flows assessment and implementation

Although the environmental water requirements are being incorporated into high-level policies for river health and catchment management, e.g. in the WFD (European Commission, 2000), in the National Water Act in Australia (Australian Government, 2007), and environmental impact assessment legislation in China (Chen and Wu, 2019), there are still significant impediments to achieve adequate effectiveness (Arthington et al., 2018b). Among them, an inadequate level of political support and stakeholder engagement, insufficient resources (e.g. funding for the technical environmental flow studies, monitoring, and management) and data (e.g. flow, geomorphological, ecological, social, and ecological data are needed for assessment and implementation), lack of knowledge, institutional barriers, and conflict of interests (Harwood et al., 2017; Le Quesne et al., 2010).

Although it is well recognised that the flow regime components and dynamism are essential to aquatic environments, most environmental flow approaches applied worldwide are centred on the preservation of low flows (Tharme, 2003). Minimum flows are usually a legal discharge value established by river basin authorities and are far from adequate for aquatic biota, as it covers only one among the several ecosystem demands (Jager and Smith, 2008). In the case of floodplain rivers, for example, environmental flow recommendations that ensure the connectivity between the floodplain and the river channel are essential, so flow components (e.g. magnitude of minimum flows, frequency and timing of high flow pulses) that maintain the connectivity, water temperature, flux of sediments and nutrients have to be considered in their environmental flow strategies (Hayes et al., 2018).

Furthermore, from a broader perspective, the usual environmental flow strategies prioritise the flow regime (i.e. hydrological alteration as the primary dam-related impact) at the expense of other environmental drivers (e.g. temperature, sediments, and connectivity; Olden and Naiman, 2010; Wohl et al., 2015). In many cases, flow restoration is insufficient to re-establish ecosystem integrity downstream of dams (Poff, 2018). In an application of the ELOHA framework, McManamay et al. (2013) advised the incorporation of additional variables besides the flow regime, such as temperature, stream gradient, and sediments to maintain crucial flow-ecology relationships, as these variables can be critical for successful fish recolonisation in regulated rivers.

Flow intermittency is also rarely considered in environmental flows, although ephemeral streams are common in Mediterranean river networks (Stubbington et al., 2018). The complexity of dealing with the effects of non-flow events on biological

communities, the temporal and spatial variability, and the absence of data are the main difficulties when dealing with temporary rivers (Acuña et al., 2020). Gauging stations are seldom located in these water bodies (<5% in Portugal and <1% in Spain; Stubbington et al., 2018) and the high spatial and temporal dynamism limit the performance of biomonitoring approaches, e.g. lack of reference values or misclassification of anthropogenic stress (Cid et al., 2020).

Organism dispersal is also a relevant process in this context, given its influence on the community structure, especially in highly dynamic ecosystems (Cid et al., 2020; Poff, 2018). The movements to find suitable habitats, colonise new sites, or dispersal as propagules create a flux of individuals, so local communities are linked together via dispersal in a metapopulation (Brown and Swan, 2010; Fagan, 2002). However, this issue is rarely considered in conservation strategies (Poff, 2018).

Thus, environmental flow science needs to incorporate a range of new elements (related or not to flow) in the environmental analysis and recommendations. This is essential as we move into a new era of dynamic, non-stationary drivers (e.g. climate change, water demand) impacting future hydrological and environmental conditions and hence the aquatic ecosystems (Poff, 2018).

1.4 Biological indicators of dam-related impacts

The use of biota to measure and track changes in running waters originated in the latter half of the 19th century in a public health context to control infectious diseases (Bonada et al., 2006). By 1900, aquatic communities (including macrophytes, macroinvertebrates, and fish) were first employed as indicators of freshwater pollution in Germany, motivated by the inability of chemical criteria to detect stressors or pollution sources in which discharged contaminants were not present (Bonada et al., 2006; Yoder and Rankin, 1998). Nowadays, the responses of several biotic groups (e.g. diatoms, phytoplankton, macrophytes, macroinvertebrates, and fishes) to changes in the stream environment, ecosystem configuration (e.g. longitudinal profile of the river; Vannote et al., 1980), and a range of anthropogenic stressors (e.g. pollution, presence of dams and impoundments, invasive species; Dudgeon, 2019) allows to establish biological monitoring (or "biomonitoring") as an essential component in the management and conservation of freshwaters and the ecosystem services they provide (Friberg et al., 2011).

The impacts of dams and the effectiveness of environmental flows and other river conservation strategies can be effectively assessed with biological communities, as riverine biota is highly diverse and offers a spectrum of environmental responses over

their life cycles (Bonada et al., 2006). For instance, in rivers affected by hydropower dams, fish were used to assess the effectiveness of bypass systems to access spawning habitat (Nagel et al., 2021). Macroinvertebrate communities were used to monitor the effects of sediment flushing from dams (Folegot et al., 2021) and macrophyte and diatom communities were employed to assess hydrological alteration effects (Goldenberg-Vilar et al., 2021).

Most biomonitoring programmes have multiple objectives and different spatial (i.e. local and regional) and temporal scales (Resh, 2008). Therefore, the use of multiple assemblages and metrics to cover different pressures is recommended (Resh, 2008; Soininen and Könönen, 2004) and increasingly reported in the literature (e.g. Hering et al., 2006; Marzin et al., 2012; Tison-Rosebery et al., 2022). For instance, the ubiquity of certain organisms, such as diatoms, insect macroinvertebrates, and some fish species, makes them good indicators of local habitat conditions, while larger fishes and non-insect macroinvertebrates might be better indicators of the network connectivity due to their water-restricted mobility (Figure 1.8; Lammert and Allan, 1999; Mangadze et al., 2016).

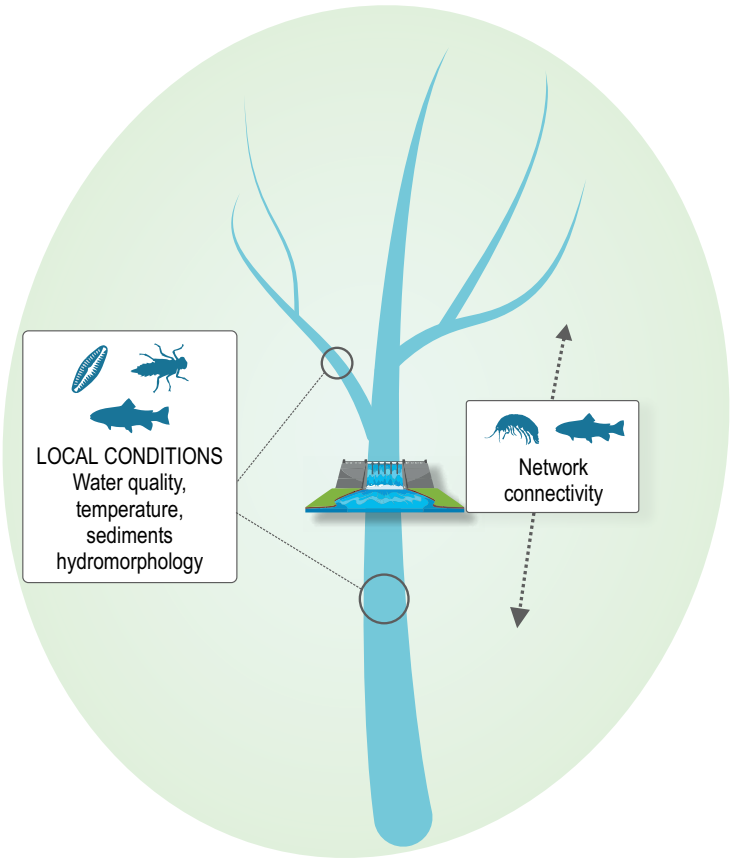


Figure 1.8 Bioindicators used to assess dam-related alterations at the local (e.g. changes in water quality, sediments, and river hydromorphology using diatoms, macroinvertebrates, and fish) and at broader spatial scales (network connectivity using non-insects macroinvertebrate and fish).

As environmental regulations are being often executed across large geographical extents (e.g. WFD in the European context), biomonitoring tools require a redefinition or rescaling to enable their application to multiple bioregions (Statzner et al., 2001). In this context, organisms' traits are a potential tool to unify taxonomic measurements and develop general, comparable rules to explain and predict ecological processes (Funk et al., 2017). Traits are defined as characteristics reflecting species' adaptation to the environment, and can be divided into biological (e.g. size, feeding habits, and reproductive strategies) and ecological traits (e.g. temperature preferences, tolerance to pollution; Menezes et al., 2010). They have been increasingly employed as a way to connect niche-based mechanisms to community patterns, transforming the modelling of species distribution and population dynamics (Cadotte et al., 2015; Dawson et al., 2021).

1.4.1 Diatoms

Diatoms (unicellular algae with siliceous cell walls) have been extensively used in the biological assessment of riverine ecosystems (Passy, 2007; Potapova and Charles, 2002; Reid et al., 1995). Their large biodiversity and presence in almost all aquatic habitats, their fundamental role in food webs and biogeochemical cycles, and their direct responses to chemical and physical changes make diatom communities valuable indicators of environmental conditions (Stevenson and Sabater, 2010).

A wide-ranging literature (e.g. in marine, coastal, and lentic habitats) emphasises the relevance of diatoms as environmental indicators (e.g. Birks et al., 1990; Krajenbrink et al., 2019; Pandey et al., 2017; Rimet and Bouchez, 2011). Moreover, in the last decade, diatom communities have also been used to investigate the effect of dams and other hydromorphological changes in rivers (e.g. Gillett et al., 2016; Goldenberg-Vilar et al., 2021; Wang et al., 2022; Wu et al., 2019b).

Several biotic indices based on diatom communities have been developed to assess water quality and pollution, such as the Biological Diatom Index (BDI; AFNOR, 2000) and the Pollution Sensitivity Index (IPS; CEMAGREF, 1982). These metrics, which are also used in the WFD water bodies' assessment, are based on the pollution sensitivity of specific diatom species. However, the accuracy of these biotic indices is hampered by the difficulty of species identification, particularly for rare species, the unstable and fast-changing diatom taxonomy, and their ecological requirements that vary across biogeographical regions (Berthon et al., 2011; Hlúbiková et al., 2014).

Therefore, the diatom guild approach developed by Passy (2007) is now frequently used as an alternative to taxonomy-based metrics. The guilds combine information on diatoms' tolerance to nutrient limitation, ability to acquire resources, and resistance

to disturbance (Soininen et al., 2016). The low profile guild is composed of small-sized diatoms under nutrient depletion dwelling within the boundary biofilm layer and, therefore, more resistant to physical disturbance. In contrast, due to their extended shape and tall stature, high profile diatoms can easily access nutrients and light but are more susceptible to perturbations (e.g. currents or grazers). The motile guild, which consists primarily of eutrophic and pollution-tolerant species, has the physical ability to seek the most suitable habitat and, therefore, is comparatively free of both resource and disturbance constraints (Passy, 2007). Finally, Rimet and Bouchez (2012) proposed the planktonic guild, which consists of diatoms adapted to lentic environments with morphological features to withstand sedimentation.

1.4.2 Macroinvertebrates

Rivers and streams contain a significant diversity of macroinvertebrates. They consist mainly of arthropods (insects, mites, crustaceans), molluscs (snails, mussels, clams), annelids (segmented worms and leeches), nematodes (roundworms), among others (Carter et al., 2017; Hauer and Resh, 2017). Most of them are benthic (i.e. associated with surfaces of the channel bottom, such as the bedrock, cobbles, sediments, or other stable surfaces) rather than free-swimming species (Hauer and Resh, 2017).

Macroinvertebrates are the organisms most often used in lotic ecosystem monitoring (Bonada et al., 2006), and their community abundance, taxonomic richness, and evenness have been routinely investigated in many water quality studies (Álvarez-Cabria et al., 2010). This is motivated by the macroinvertebrates' ubiquity, well-known taxonomy, the large number of species sensitive to perturbations, and easy sampling (Carter et al., 2017).

Macroinvertebrate biotic indices are also largely used to measure organic pollution. As an example, the Biological Monitoring Working Party (BMWP; Armitage et al., 1983) was developed in the United Kingdom for a national assessment of river pollution and takes into account the tolerance of all benthic macroinvertebrate families in a sample. The BMWP has been and is widely used in Europe, with national or regional adaptations to account for new families and their sensitivity ratings, e.g. in Iberian countries (IBMWP; Alba-Tercedor et al., 2002). Similarly, the Averaged Score Per Taxon (ASPT) computes the average of all macroinvertebrate families' tolerance scores, however, it does not depend on the family richness as BMWP.

Traditional taxonomic approaches also include diversity indices to assess macroinvertebrate community composition and structure. The Shannon-Wiener

index accounts for the species richness and relative species abundances, reflecting the dominance and structure of the community (i.e. higher values indicate that all species exhibit the same relative abundance; Feld et al., 2014). The Simpson diversity also considers the species richness and their relative abundances, but the score represents the likelihood that two individuals in a sample do not belong to the same species (i.e. higher values indicate a more diverse community; Feld et al., 2014).

In contrast to most biotic indices analysing water quality and pollution, the Lotic-invertebrate Index for Flow Evaluation (LIFE; Extence et al., 1999) links the riverine macroinvertebrate communities to the prevailing flow regimes. This metric was mostly applied in the UK and is intended for assessing the impacts of variable river flow regimes on macroinvertebrate communities (Extence et al., 1999; Laini et al., 2018).

1.4.3 Fish

Over 10,000 fish species live in freshwaters, accounting for at least 40% of all fish species (Lundberg et al., 2000). Fish communities are highly dynamic, with their distribution, composition, and structure varying across time and space (Matthews, 1998).

When assessing environmental changes, fish communities provide several advantages in comparison to other biotic groups. They are vulnerable to a range of stressors due to their dependence on the habitat components such as sediment, vegetation, and water temperature for spawning, feeding, and survival. Furthermore, because of their longevity, the effects of disturbances along different life stages can be observed, providing long-term records of environmental stress (Fausch et al., 1990). The migratory movements of some species along the river network can indicate the status of habitat connectivity and fragmentation, e.g. anadromous salmonids or catadromous eels perform long migrations, while other species make short movements to access spawning microhabitats or refuge (Matthews, 1998). Moreover, the long tradition of ecological, physiological, and ecotoxicological research on fish has contributed to advance in the knowledge of a large number of species and their ecological requirements, making them good indicators for changes in ecological conditions (Chovanec et al., 2003; Schiemer, 2000).

Several local fish-based biotic indices have been developed to assess riverine ecosystem integrity at the European level. However, the high degree of basin-specific assemblages, the lack of information on specific species, the marked intra- and inter-annual variability in fish communities, and the scarcity of pristine streams (i.e.

reference conditions) hamper the development of metrics valid across larger spatial scales (Aparicio et al., 2011; Ferreira et al., 2007; Segurado et al., 2014). In this context, the European Fish Index (EFI+; EFI+ Consortium, 2009) was the first standardised fish-based assessment method developed to be applied across European rivers (Segurado et al., 2014). This multimetric index quantifies the deviation from fish assemblages in reference conditions based on functional guilds describing the main ecological and biological fish characteristics (EFI+ Consortium, 2009).

1.5 The Spanish case study

Spain is a country located in southwestern Europe, covering 505,990 km^2 (Figure 1.9; Tapiador, 2020). It is bordered by France to the north along the Pyrenees and the Bay of Biscay, by Portugal and the Atlantic Ocean to the northwest, and by the Mediterranean Sea to the south and east. On its southern extreme, a 13-km strait separates it from the African continent (Tapiador, 2020).

The combination of its geographical location, climate, and relief makes Spain a biogeographically complex country in Europe. The water availability in Spain (around 40,000 $hm^3.year^{-1}$) is relatively high; however, it is irregularly distributed across time and space due to the country's geographic situation, relief, and the strong climatic contrasts (Costejà et al., 2002; Ministerio de Medio Ambiente, 2004).

The mainland is composed of a complex relief configuration (Figure 1.9). A high interior plateau, the Meseta (around 600 m.a.s.l.) is divided by the Central System into the northern (800-850 m.a.s.l.) and the southern sub-plateau (500-700 m.a.s.l.). The Meseta is surrounded by the Cantabrian Cordillera (2,600 m.a.s.l.) to the north, the Iberian System (2,300 m.a.s.l.) to the east, and the Sierra Morena (1,300 m.a.s.l.) to the south. Outside this central plateau, the Pyrenees and the Betico System reach up to 3,400 m.a.s.l (Rivas-Martínez et al., 2004; Suárez-Seoane et al., 2002). The geology is, in general, characterised by siliceous rocks in the west and calcareous formations in the east of the Iberian Peninsula (Sabater et al., 2022).

The Atlantic Ocean and the Mediterranean Sea exert a strong influence on the Iberian Peninsula's climate (Figure 1.10). The country's north and west parts are largely exposed to the maritime Atlantic winds, with mild summers and wet winters (e.g. Vigo and Santander in Figure 1.10). The mountain areas present cold climates and abundant precipitations. The southern part is the hottest and driest region, as it receives warm air masses from the Mediterranean Sea and the Sahara Desert (e.g. Cádiz and Murcia in Figure 1.10; AEMET and IMP, 2011).

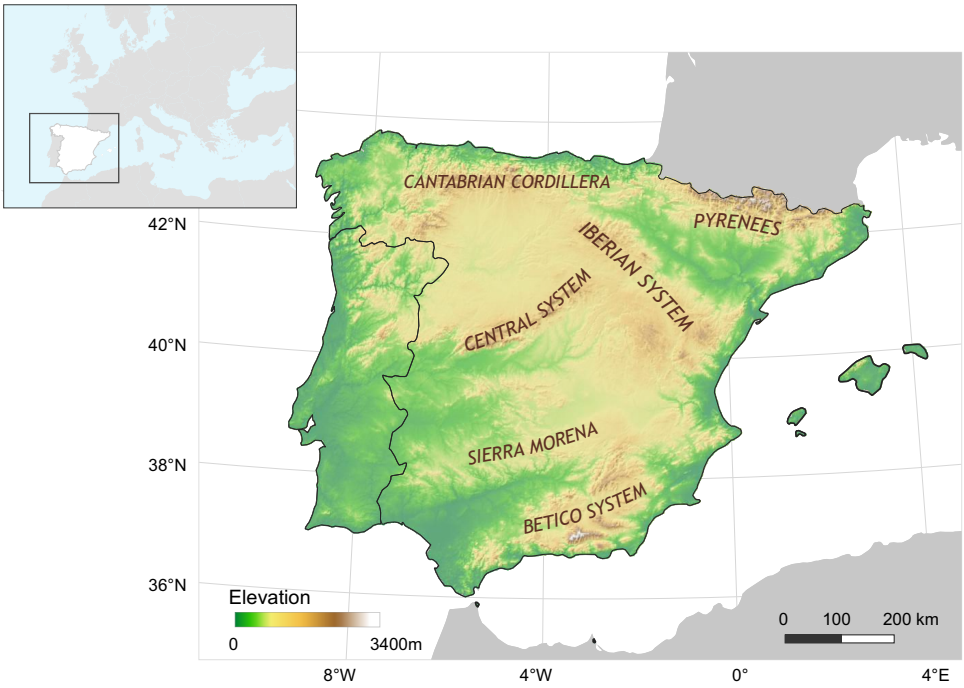


Figure 1.9 Location of Spain in Europe, its orography, and the main geographical accidents.

The precipitation (Figure 1.10b) has a marked spatial gradient, with humid zones coinciding with mountain headwaters (Cardoso Pereira et al., 2020). Rainfall is concentrated in the cold season in the Atlantic regions and in the spring and autumn in the Mediterranean areas (López-Moreno et al., 2008).

The vegetation also reflects the climatic diversity present in the country. The phytoclimatic regions determined by Allué (1990; Figure 1.11) describe the wide range of bioclimatic conditions found in the country, as they link climatic conditions and potential vegetation coverage (Novillo et al., 2019).

In the mountain ranges (oroboreal phytoclimates represented by pink and purple tones in Figure 1.11), vegetation is characterised by conifers, e.g. the *Pinus uncinata* in the Pyrenees, the *Pinus sylvestris* in the Central System and Teruel mountain ranges, and alpine and sub-alpine grasslands (Bedia et al., 2014; Tapiador, 2020). Broad-leaf deciduous forests are present in Atlantic zones with high precipitation and mild temperatures (nemoral phytoclimates; green to blue tones in Figure 1.11), e.g. the gall oak (*Quercus faginea*) in humid and cold areas, the black oak (*Quercus pyrenaica*) in less humid mountain areas throughout the north of the country, and the beech (*Fagus sylvatica*) in cool and humid mountain areas (Bedia et al., 2014; Tapiador, 2020). In the semi-arid regions in the south and the Ebro depression (genuine Mediterranean, yellow tones areas in Figure 1.11), the kermes oak (*Quercus coccifera*) and the holm oak (*Quercus ilex*) can be found. In very dry regions (orange

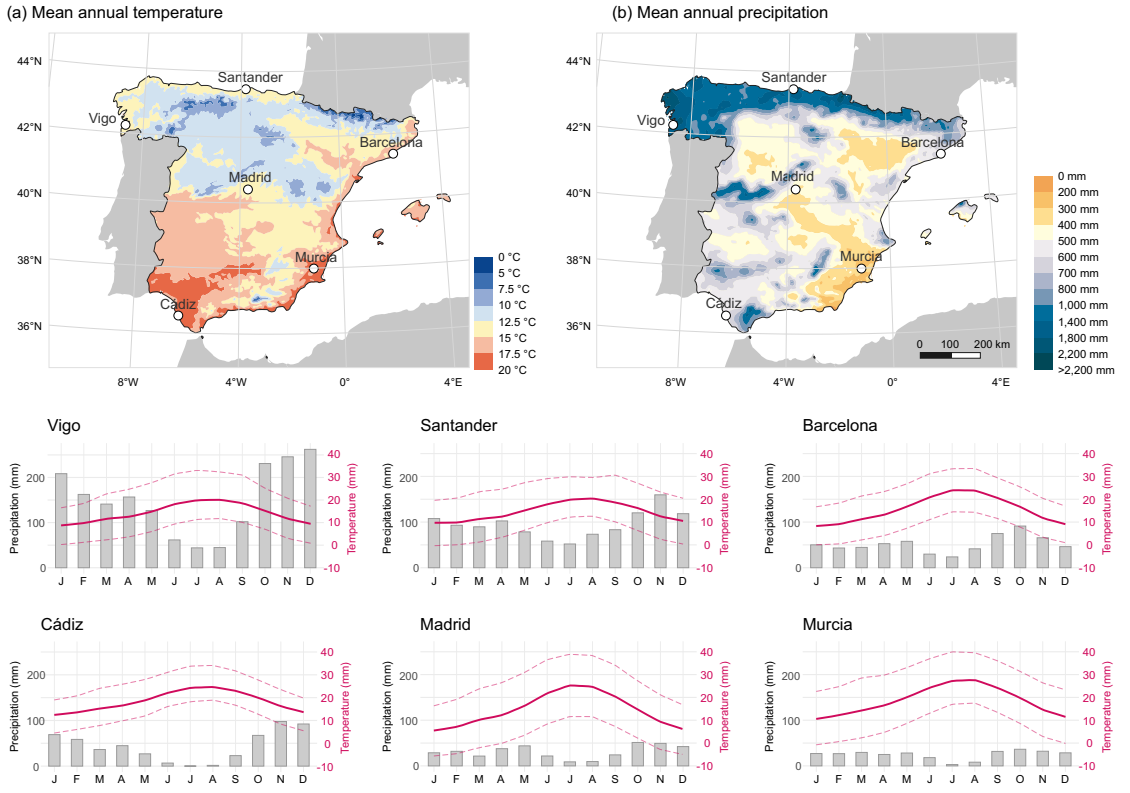


Figure 1.10 (a) Mean annual temperatures and (b) mean annual precipitation in Spain (1981-2010). Data source: IGN (2019c). Mean monthly precipitation and temperature (1981-2010; dashed lines represent minimum and maximum temperatures) in six Spanish cities. Data source: AEMET Open data (https://opendata.aemet.es).

tones in Figure 1.11), shrubs like the wild olive (*Olea europaea*) and the mastic tree (*Pistacia lentiscus*) are predominant (Bedia et al., 2014).

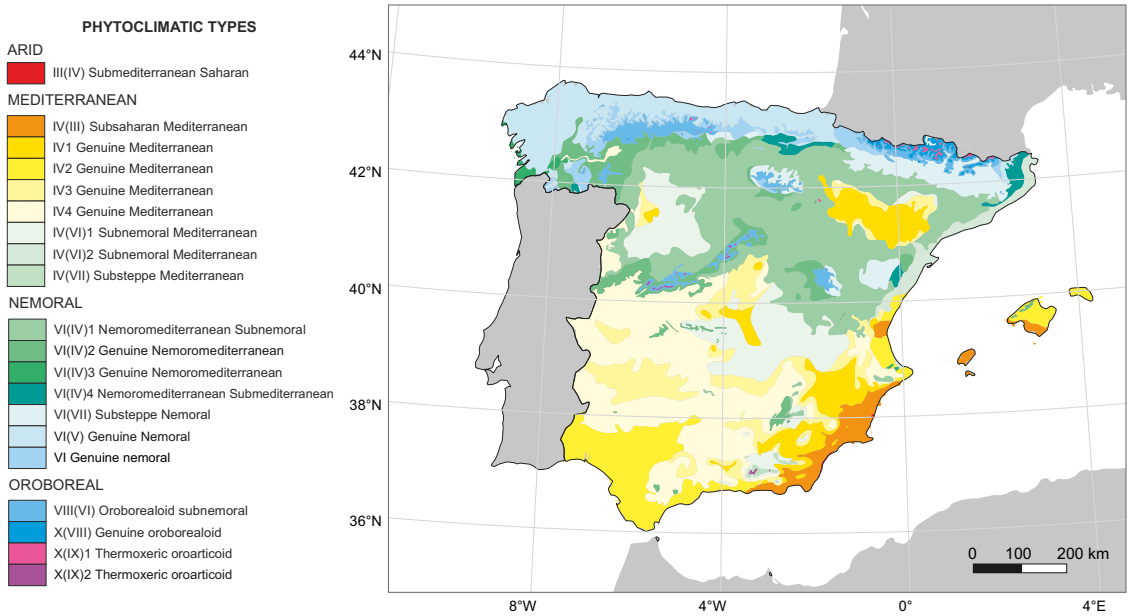


Figure 1.11 Phytoclimatic regions in Spain according to Allué (1990). Source: IGN (2019b).

1.5.1 Spanish river network and the main rivers

The Spanish river network (Figure 1.12) is divided between Atlantic basins (i.e. discharge into the Atlantic Ocean), covering around 69% of the Iberian Peninsula, and the Mediterranean basins (i.e. discharge into the Mediterranean Sea) covering the remaining 31% of the Peninsula (Ministerio de Medio Ambiente, 2004; Sabater et al., 2022).

The contrasting climatic differences, the geographic situation, and relief influence deeply the water availability along the Spanish river network (Figure 1.12). Mediterranean rivers have a rainfall-based flow regime, reaching their maximum discharge in spring and autumn and minimum in summer. Atlantic rainfall-based rivers have higher inputs, with peak flows in late winter and minor decreases in their summer flows. Rivers with a nival regime (e.g. in the Pyrenees or other mountain ranges) reach the maximum discharge levels in spring and keep a relatively constant flow in summer (Sabater et al., 2022). The southern and central Mediterranean basins (Guadiana, Guadalquivir, Segura, and Júcar; Figure 1.12) cover 41% of the territory, concentrating 37% of the population but only 19% of the total available water resources. In contrast, the northern river basins cover 59% of the area but detain 81% of the water resources (Costejà et al., 2002).



Figure 1.12 Spanish river network and the main river basins. Data source: MITECO (2022b) and EU-Hydro (European Environment Agency, 2015).

In the north of the Iberian Peninsula, small mountain rivers run from the Cantabrian Cordillera to the Bay of Biscay. The valley of the Miño and Sil rivers, which flow westward to the Atlantic Ocean, is located at the western border of the Cantabrian Cordillera (Viveen et al., 2013). The Duero, Tagus, and Guadiana rivers flow over the Meseta and are enclosed by the Cantabrian Cordillera, the Iberian System, and the Sierra Morena, reaching the Atlantic Ocean from the western coast (Figure 1.12). The Ebro river, which is the largest river from the Mediterranean basins, borders the Meseta, reaching the Mediterranean Sea from the eastern coast, along with the Júcar, Segura, and other small Mediterranean basins (Ministerio de Medio Ambiente, 2004; Sabater et al., 2022).

The Duero (*Douro* in Portuguese) is the third largest river in Spain in length (927 km in total: 597 km in Spain and 330 km in Portugal) and the largest river basin in area (Table 1.1; 80% of the area is located in Spain). The climate is mainly Mediterranean, with a continental climate influence in the east and an Atlantic, milder climate in its Portuguese portion. Most of the catchment is covered by medium-sized cities (León, Burgos, Valladolid; <300,000 inhabitants) and small villages (<1,000 inhabitants), croplands (wheat, oats, barley, vineyards) concentrated mainly in the central region, and forests in the headwaters (Sabater et al., 2022).

The Ebro river is one of the largest Iberian rivers (910 km), flowing from the Cantabrian Cordillera to the Mediterranean Sea. The contrasting climatic and hydrological patterns in the basin (i.e. mean annual precipitation varying from 307 to 2,451 mm.year^{-1}) is a consequence of the heterogeneous topography, characterised by mountain ranges enclosing the Ebro Valley (López-Moreno et al., 2011). The high runoff in the mountain headwaters motivated the construction of numerous dams between the 1950s and the 1980s, reaching a storage capacity of around 3,000 hm^3 (i.e. half of the mean annual runoff of the Pyrenean rivers), which attends to 77% of the irrigation water demands and generates 62% of the hydropower produced in the Ebro basin (López-Moreno et al., 2008).

The Tagus (*Tajo* in Spanish, *Tejo* in Portuguese) is the longest river in the Iberian Peninsula, flowing through 1,007 km from the Sierra de Albarracín (east-central Spain, 1,590 m.a.s.l.) to the Atlantic Ocean in Lisbon (Sabater et al., 2022). The catchment provides water for irrigated agriculture (the main water user, with 2,300 km^2), urban supply (to about 15% and 30% of the Spanish and Portuguese population, respectively, including Madrid and Lisbon metropolitan areas), industry (cooling of thermal plants), and fishery activities in the Tagus estuary (López-Moreno et al., 2009). Moreover, a part of the discharge is transferred to the Segura basin (650 hm^3.year^{-1}; Sondermann and de Oliveira, 2022). The basin is highly regulated, and the Alcántara reservoir (storage capacity of 3,162 hm^3, the second largest in

Europe) causes important changes in the hydrological regime and drought patterns (López-Moreno et al., 2009).

The Guadiana River is located in the southwest of the Iberian Peninsula and flows over 810 km (550 km in Spain, 150 km in Portugal; Sabater et al., 2022). The basin experiences severe hydric stress due to the Mediterranean climate (hot and dry summers and precipitations concentrated between October and April), frequent drought events, the growing water demands (irrigated agriculture, tourism, industrial uses in the Huelva region in the Spanish part), and the overexploitation of groundwater resources (Bromley et al., 2001).

The Guadalquivir river (657 km long) is mainly located in the Andalusia region. The basin is formed by a geographic depression delimited by the Sierra Morena in the north, the Betico System in the south, and the Atlantic Ocean in the southwest (Sabater et al., 2022). Agriculture is an important economic activity in the basin (8,560 km^2 of irrigated lands) and the main water user (2,741 hm^3 in 2015, corresponding to 82% of the total water consumption; CHG, 2015) in the basin. The region is highly vulnerable to climate change, and the low water availability in this semi-arid region led to the construction of several dams and reservoirs, especially in the northern Guadalquivir tributaries, as well as the adoption of more efficient irrigation systems (Rodríguez Díaz et al., 2007; Romero-Jiménez et al., 2022).

The Júcar and Segura basins are located in the eastern of the Iberian Peninsula. Drought events are common in both basins due to the semiarid climate and the high hydrological variability (Carmona et al., 2017; Miró et al., 2021). The high water demands motivated by the large population density (especially in the Júcar basin, see Table 1.1), tourism, intensive irrigated agriculture (3,900 km^2 in the Júcar basin and 2,624 km^2 in the Segura basin), and livestock make them highly vulnerable to water stress and the climate change impacts (CHJ, 2015; CHS, 2015). Since 1979, the Segura basin receives water transferred from the Tagus headwater to supply urban and agricultural demands (on average, 350 $hm^3.year^{-1}$), which generated socio-economical development in the region but also water use conflicts (Senent-Aparicio et al., 2021).

Table 1.1 General characteristics of the largest rivers in Spain and their catchments. Source: Sabater (2008) and CHMS (2022).

	Duero	Ebro	Tagus	Guadiana	Guadalquivir	Júcar	Segura	Miño-Sil
Catchment area (km^2)	97,290	85,362	80,600	67,048	57,527	21,208	19,182	17,581
Mean annual discharge (hm^3)	13,560	13,410	9,930	6,180	7,220	810	820	7,660
Mean catchment elevation (m)	874	770	599	504	566	817	644	738
Mean annual precipitation (mm)	665	672	599	532	521	448	398	1,183
Mean air temperature (°C)	11.4	11.4	13.8	15.2	15.5	13.1	14.4	11.4
Urban land uses (% of catchment)	0.7	0.6	1.7	0.6	1.1	0.7	1.7	1.3
Arable terrain	55.1	47.1	45.6	68.4	62	51.6	53.4	25.1
Pastures	1	1.9	0.3	0.1	0	0	0	2.9
Forest	17.5	22.3	21.5	7.8	12.8	17.2	14.6	15.4
Natural grasslands	23.5	25.5	28.8	21.8	20.9	29.8	24.9	17.2
Number of large dams (≥15 m)	75	70	72	87	55	13	15	51
Population density (inhabitants . km^{-2})	37	34	136	24	69	207	78	45

The Cantabrian rivers are located in a narrow area in the north of the country, with headwaters in the Cantabrian Cordillera and draining into the Cantabrian Sea. The area is characterised by numerous short rivers constrained in narrow valleys and with relatively high water flows, and their temperate oceanic climate (mild winters, warm summers and abundant rainfall throughout the year) is a result of the proximity to the ocean and the northwest winds (Sabater et al., 2022). In total, 28 main rivers cover a total surface of 23,223 km^2 (Nalón, Navia, and Nervión among the largest basins). Water uses are mostly domestic (around 67% of the total water consumption) and industrial (22%), and dams are used mainly for small-scale hydroelectric generation (CHC, 2015; CHC and URA, 2015). Woodlands, meadows, and grazing and agriculture are the main land uses; urban areas and industry are mostly concentrated in the eastern part of the coast, where population density is also higher (Díez et al., 2000; Prego et al., 2008).

The Miño-Sil river basin covers a relatively small area in the northwest of the country, yet it has a high average discharge (Table 1.1). The climate has a strong Atlantic influence, with mild temperatures and abundant rainfall throughout the year (ranging from 700 to 1,900 $mm.year^{-1}$). The agricultural sector is the main water user (67% used for irrigation, 6.4% for livestock), followed by urbanisations (16.1%) and industries (8.4%). The catchment is regulated by more than 70 large dams (>10 m), with Belesar (654.1 hm^3), As Portas (535.7 hm^3), Barcena (340 hm^3) among the largest ones (CHMS, 2022).

Finally, smaller, intra-regional basins (i.e. flowing entirely within an autonomous community), such as the Andalusian Mediterranean basins (17,762 km^2; Junta de Andalucia, 2015), the internal basins of Catalonia (16,438 km^2; Agència Catalana de l'Aigua, 2022), and Galician Coast (12,991 km^2; Augas de Galicia, 2023) also compose the Spanish river network.

1.5.2 Water uses in Spain

In Spain, agriculture and livestock account for 78.8% of the total water consumption, while urban water supply corresponds to 17.3% and industrial uses to 3.9% (Fornés et al., 2021). With an irrigated area of 38,779 km^2 in the country (MAPAMA, 2021), the water consumption for irrigation is massive (estimated at 15,495 hm^3 in 2018, from which nearly 75% is derived from surface waters; INE, 2020) and mostly concentrated in the Mediterranean basins (Figure 1.13). Irrigated lands generate 60% of the total agricultural production and 80% of the agricultural exports in the country, representing an essential and prosperous economic activity in Spain (Berbel et al., 2019; MAPAMA, 2021).

The groundwater abstraction (around 7,000 $hm^3.year^{-1}$) corresponds to 22% of the total water demands in Spain and supplies water for about 13 million inhabitants (28% of the population) and around 27% of the total irrigated area (De Stefano et al., 2015). The highest shares of groundwater abstraction (relative to the total river basin abstraction) are in the Canary and Balearic Islands, Júcar, Catalan, and Andalusian river basins (De Stefano et al., 2012; Figure 1.13). In many regions, groundwater is a strategic water resource, especially during droughts when surface water is scarce, e.g. in Madrid (Béjar-Pizarro et al., 2017); however, the main use of groundwater is in the agricultural sector (De Stefano et al., 2015). The management of these groundwater resources is a complex task and some of the aquifers, especially in the south of the country, are considered overexploited (Fornés et al., 2005). For instance, in the Guadiana basin, the overexploitation of groundwater resources since the 1970s for irrigation led to a long-term reduction of the water table and available water resources, causing the degradation of river and wetland ecosystems (Bromley et al., 2001).

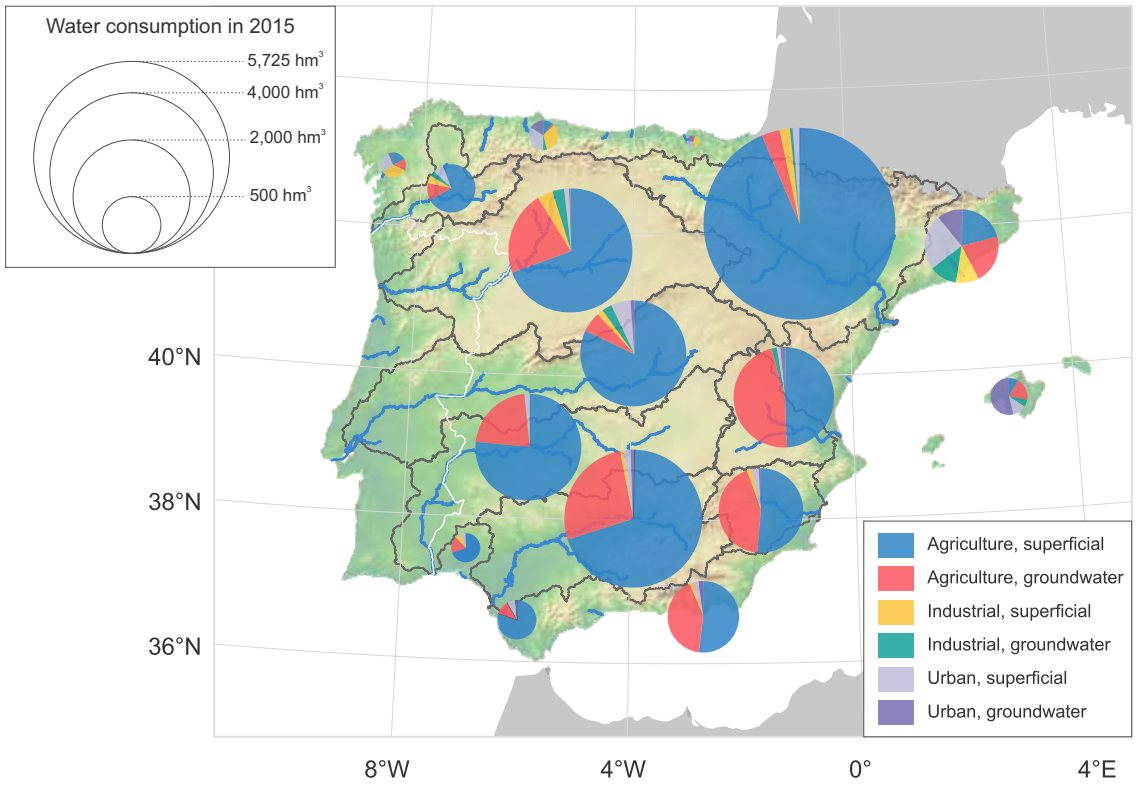


Figure 1.13 Water uses in 2015 in the main Spanish river basins. Adapted from IGN (2019a).

The construction of dams and reservoirs increased rapidly in Iberian rivers. For more than three decades following the Spanish Civil War (1936-1939), the Francoist regime promoted dam and reservoir construction. In 1940, reservoirs in Spain had a

total storage capacity of 4,000 hm^3; by 1975, this capacity increased to 37,000 hm^3, with more than 800 dams (Fornés et al., 2021).

Nowadays, despite the country's relatively small size, Spain is among the top ten dam-building countries worldwide, with 1064 large dams (height ≥15 m or impounding more than 3 hm^3; ICOLD, 2021). The total river runoff in the country is around 111,000 hm^3 per year, and the nominal reservoir capacity is ca. 56,069 hm^3 (MITECO, 2022a), with La Serena (3,220 hm^3 capacity, Zújar river), Alcántara (3,162 hm^3, Tagus river), and La Almendra (2,649 hm^3, Tormes river in the Duero basin) as the largest reservoirs in Spain (IGN, 2019a).

The relatively high percentage of water storage in the country is essential to deal with dry climatic conditions and irregular rainfall (Fornés et al., 2021). These infrastructures increased significantly the water availability in southern basins in Spain, attenuating the impact of reduced streamflow and the uncertainties of climate change projections (Granados et al., 2021). For instance, in the Guadiana basin, the reservoir La Serena (the largest one in Spain and third in Europe, see Figure 1.14), increased the water volume stored over 40 times than that obtained under natural flow conditions (Ministerio de Medio Ambiente, 2004).

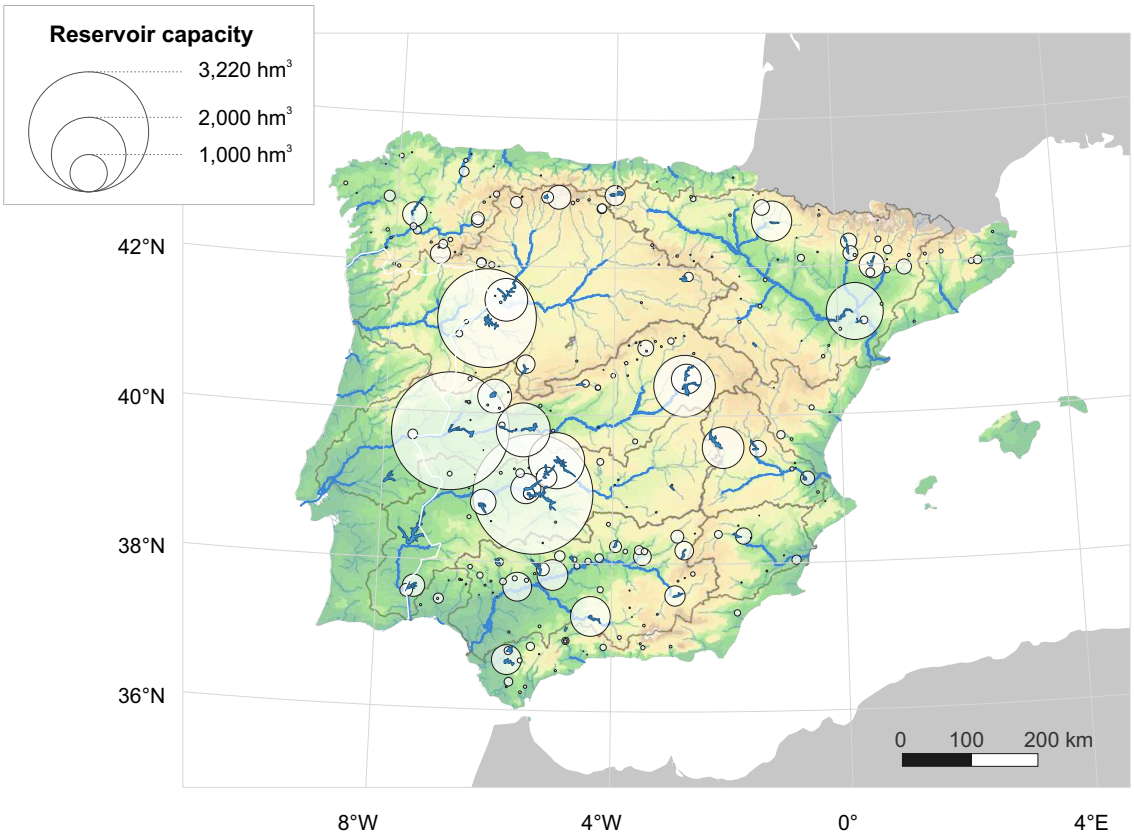


Figure 1.14 Distributions of the main reservoirs in Spain and their capacity. Adapted from IGN (2019a).

The construction of dams causes changes in the flow seasonality, intra-annual variability, and minimum and maximum flows (Peñas and Barquín, 2019). In general, in the wet season the flow is less variable, the low flows frequency increases, while the frequency of high flow events decreases. In the dry season, the opposite occurs: the flow variability increases, low flow events are less frequent, while the high flows are more frequent (Mezger et al., 2021). However, the patterns of flow alteration may also depend on the natural hydrological conditions and pre-dam regimes (Peñas and Barquín, 2019).

1.5.3 Current state of Spanish rivers and the e-flow management

In 1985, with the Water Act in Spain, the development of river basin management plans become a legal requirement (Willaarts et al., 2014). Then, with the incorporation of the WFD into Spanish environmental law in 2003, the conservation of the aquatic environment became one of the priorities of the water policy. The Spanish Water Agencies were then responsible for elaborating the hydrological plans, setting environmental protection objectives, managing the surface water and groundwater resources, infrastructures (e.g. dams, channels), monitoring the water bodies' flow regime, quality, and ecological status (Font and Subirats, 2010; Román-Barreiro, 2018).

As part of the Directive, water bodies were grouped according to their physical and morphological attributes (e.g. altitude, water conductivity, geology, and catchment size), contributing to more specific and accurate ecological assessments (Hering et al., 2010). Spanish rivers were classified in a total of 37 river typologies (e.g. karstic Mediterranean rivers, coastal Mediterranean rivers, humid siliceous mountain rivers, siliceous Cantabric-Atlantic river axes) and their corresponding biological, hydromorphological, and chemical reference conditions (i.e. conditions based on sites with minimal changes and the absence of significant human pressures; Wallin et al., 2003) were established (CEDEX, 2004; BOE, 2005). This step was challenging given the high environmental variability and anthropogenic interference in the Spanish rivers and the unclear concept of what the minimally-disturbed conditions represented within the WFD (Pardo et al., 2012).

After the first river basin management cycle (2009-2015), the second RBMPs indicated that 51% of the surface waters in Spain were in good or very good ecological status (MITECO, 2018). Rivers, the most abundant water bodies in Spain (length over 70,000 km, 19% considered heavily modified), had 55% of their water bodies classified as good or higher ecological status. The situation was more critical in lakes

and wetlands (around 1,170 km^2, 16% considered heavily modified) and transitional waters (around 985 km^2, 60% considered heavily modified), as only 45% and 46% of the water bodies, respectively, were in good or higher ecological status. In contrast, coastal waters (around 22,200 km^2, 2% considered heavily modified), which correspond to waters located up to one nautical mile from the coastline, had 78% of their water bodies in good or higher ecological status (European Commission, 2000; European Environment Agency, 2018b).

The overall ecological status of surface waters varied spatially throughout the country (Figure 1.15). In general, the most challenging areas were the Duero (79% of water bodies in poor condition), Guadiana, and Catalan basins. The northern water bodies (i.e. the Cantabrian, Galicia, and Miño-Sil basins) presented the highest ecological status (around 70% of the water bodies in good status; Willaarts et al., 2014). However, this data needs to be interpreted with caution, as the assessment was not homogeneous across the country: there are still water bodies that have not been evaluated (2% of rivers and lakes, 5% of transitional and coastal waters; European Environment Agency, 2018b), and the majority of the ecological assessments were based solely on macroinvertebrates and diatoms (fish communities assessed in 8% of water bodies; river continuity in 18%; only two of 17 basins have developed metrics and reference conditions for these elements; European Environment Agency, 2018b; Willaarts et al., 2014).

The Spanish water legislation (ARM/2656/2008; BOE, 2008) requires the establishment of environmental flows as part of the RBMPs, with the maintenance of the aquatic ecosystem integrity downstream of dams as their main goal. The environmental flows are defined based on the minimum and maximum flows and their temporal distribution, rate of change, and high flow events (i.e. short-duration events such as bankfull discharges). The minimum and maximum environmental flows are then determined by combining hydrological and habitat simulation methods (usually for fish species in two different life stages; BOE, 2008).

The implementation of e-flows in the country, however, is mainly based on the minimum flow values (73% of water bodies), whereas the other variables are taken into account in less than 8% of the water bodies (Mezger et al., 2019). The high flow events and the rate of change are key variables in maintaining the integrity of aquatic ecosystems (Bunn and Arthington, 2002; Poff and Zimmerman, 2010). High flow pulses and flood events shape the hydromorphological characteristics of the river channel and the stream bed substrate, restore water quality after prolonged low flow periods, flush away pollutants and invasive species, and maintain the river connectivity (e.g. lateral connectivity to floodplains; Richter and Thomas, 2007).

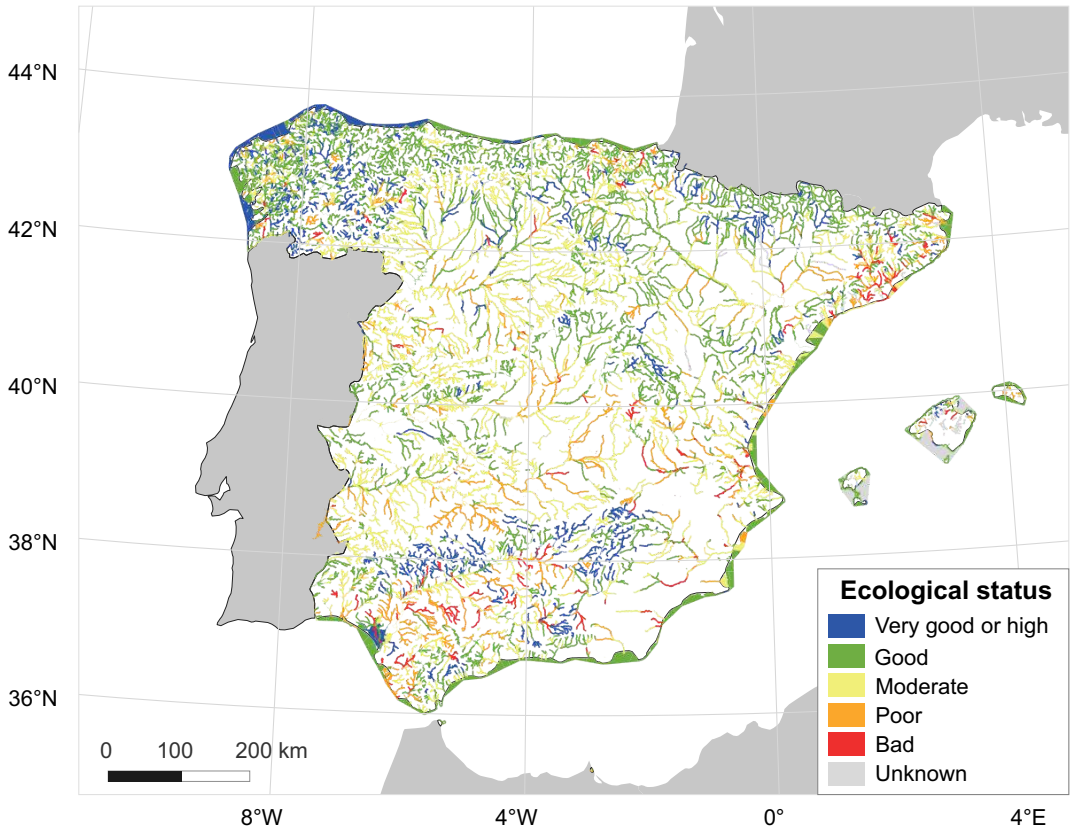


Figure 1.15 Overview of the water bodies in Spain and their ecological status in the second RBMPs (2015-2021). Data source: MITECO (2016).

1.6 Objectives of the thesis

The central aim of this PhD thesis is to assess the spatial variability patterns of biodiversity in Spanish streams and rivers and how dams and reservoirs modify these patterns. To achieve this, it is necessary to separate the natural biodiversity variability arising from natural gradients and factors from the variability introduced by stressors (i.e. dams).

This work can be divided into two parts: Chapters 2 and 3 aim to explore the natural variability of riverine communities at large spatial scales, while Chapters 4 and 5 explore how dams with different purposes affect riverine communities based on previous chapters' insights. The specific objectives are explored in the following thesis chapters:

- Assess the contribution of abiotic factors (e.g. environmental and hydrological factors) on the diatom, macroinvertebrate, and fish communities' variability in near-pristine streams under natural flow conditions throughout Spain (Chapter 2).

- Investigate the balance between the local (i.e. niche) and spatial (i.e. dispersal-related processes) variables on structuring diatom and fish communities in near-pristine streams under natural flow conditions throughout Spain (Chapter 3).

- Assess the impact of dams on diatom, macroinvertebrate, and fish communities and identify the main changes produced by hydrological and physico-chemical factors on mountain streams (Chapter 4) and in a control-impact study design (Chapter 5) in northern Spain.

- Compare the flow regulation effects on riverine biota associated with dams operated for irrigation and hydropower generation (Chapter 5).

- Compare the use of multiple biotic groups (i.e. diatom, macroinvertebrate, and fish communities) as bioindicators in terms of sensitivity to hydrological alteration and environmental factors, dispersal-related processes, and spatial scales.

- Evaluate the benefits and drawbacks of using species traits in the context of biomonitoring and conservation of freshwater ecosystems.

1.7 Structure of the thesis

This thesis is composed of six chapters. The Introduction addresses the key topics related to freshwater ecosystems, their biodiversity and its threats, the effects of dams on the flow regime and riverine biota, mitigation measures, among other relevant topics. Chapters 2 to 5 (see Figure 1.16) explore the specific objectives (as listed in the previous section), which arise from the issues and research gaps identified in the Introduction. A brief synopsis of the investigation carried out in each chapter is described below:

Chapter 2: Large-scale factors controlling biological communities: an insight into global change effects on river ecosystems

In this chapter, we investigated the key environmental gradients associated to the spatial variability of freshwater biota. Only diatom, macroinvertebrate, and fish communities surveyed in streams in good ecological and chemical status and free of other anthropogenic pressures were selected. Using redundancy analysis with forwarding selection and RLQ analysis, the landscape-scale dynamic (climate, land use and land cover, and hydrology) and static (geology and topography) environmental variables significantly contributing to communities' taxonomic and trait-based variability were identified in the three biotic groups.

Chapter 3: Large-scale spatial patterns of riverine communities: niche versus geographical distance

In this chapter, the role of environmental and dispersal-connectivity controls on biological communities' variability was investigated using distance-decay relationships and variation partitioning. To reduce the effects of other possible stressors, only communities in streams under near-pristine conditions were selected. Diatoms and fish, the two selected biotic groups, have marked differences in body size and dispersal abilities. The analyses were carried out on the taxonomic and functional community structure of both biotic groups.

Chapter 4: Irrigation dam effects on riverine biota in mountain streams

In this chapter, our main objective was to assess the effects of dams on riverine communities. To reduce the confounding effect of the environmental variability and other stressors, we selected biological communities surveyed in mountain areas in northern Spain (Cantabrian Cordillera, Iberian System, and Pyrenees) under natural or altered flow conditions (i.e. located downstream of dams). We used two-way ANOVA and RLQ analysis to assess the effects of dams on hydrology, physico-chemical variables (e.g. water temperature, conductivity, nutrients), and diatom and macroinvertebrate communities.

Chapter 5: Assessing the effects of irrigation and hydropower dams on river communities using taxonomic and multiple trait-based approaches

In this chapter, our main objective was to assess dam-related effects on diatom, macroinvertebrate, and fish communities and identify key ecological responses associated with flow regulation caused by irrigation and hydropower dams. Biological communities were surveyed in 8 control and 11 impacted streams over three consecutive years. In addition to traditional biotic indices based on the taxonomy of these communities, we assessed community-level responses using trait-based analyses with RLQ and fourth-corner methods.

Finally, the General Conclusions and Future Research are described in the last chapter, followed by References and Annexes A and B.

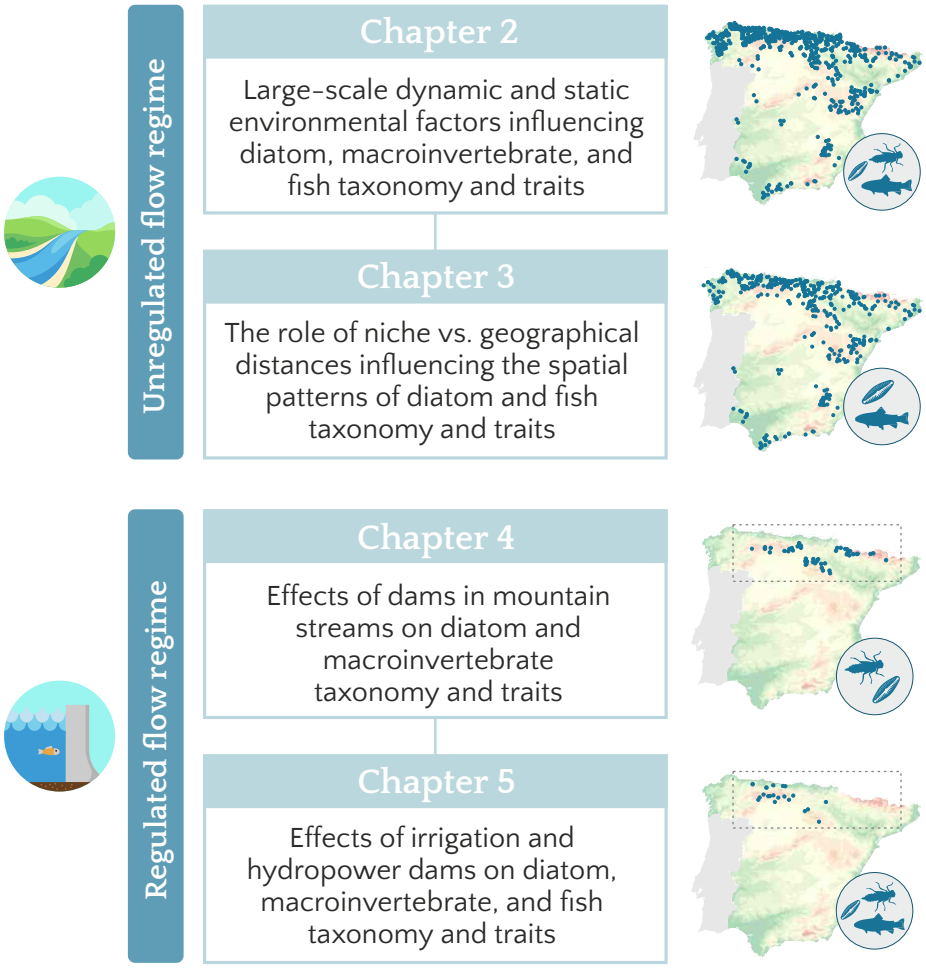


Figure 1.16 Thesis structure, divided into four main chapters, which explore freshwater biological communities under natural (Chapters 2-3) and regulated flow regimes (Chapters 4-5). Maps on the right side indicate the spatial distribution of samples in each chapter.

Chapter 2

Large-scale factors controlling biological communities: an insight into global change effects on river ecosystems

Abstract

The ongoing global environmental change poses a serious threat to rivers. Comprehensive knowledge of how stressors affect biota is thus critical to support effective management and conservation strategies. We evaluated the major gradients influencing spatial freshwater biodiversity variability in Continental Spain using landscape-scale environmental variables representing climate, land use and land cover (LULC), flow regime, geology, and topography. We selected diatom (n=117), macroinvertebrate (n=441), and fish (n=264) communities surveyed in near-pristine streams. In the three biotic groups, redundancy analysis was used to identify the environmental factors significantly contributing to communities' variability and RLQ analyses to assess trait-environment associations. The four environmental variable groups contributed to defining the major community change gradients (e.g. mountain-lowland). Siliceous, steep streams and increased precipitation levels favoured stalked diatoms, macroinvertebrates with aquatic passive dissemination, and fish performing migration. These traits were replaced by adnate, pioneer, and motile diatoms, small-sized macroinvertebrates, non-insects, and non-migratory

fish in lowland streams with warmer climates, calcareous geology, agricultural activities, and stable flow regimes. Overall, landscape-scale environmental variables showed important contributions to fish communities' variability, while diatoms and macroinvertebrates might be more related to local-scale characteristics (e.g. water quality). The upslope gradient on river networks (e.g. changes in slope, temperature, and human land uses) was common to the taxonomic and trait-based spatial variability in the three biotic groups. This result indicates that the global change effects detection in riverine biodiversity could emerge as changes in their longitudinal distributions along river networks, and implementing management actions focusing simultaneously on water temperature and hydrological regime conservation (e.g. tackling LULC changes) might be the best strategy for mitigating global change effects on rivers.

2.1 Introduction

Rivers provide essential goods and services for human and societal well-being (Baron et al., 2002). However, over the past century, an accelerated human-driven environmental change, referred to as "global change", has been impairing both biotic and abiotic components and processes on Earth (Steffen et al., 2005) and heavily threatening freshwater ecosystems (Dudgeon, 2019; Vörösmarty et al., 2010). Multiple anthropogenic pressures on rivers (e.g. water pollution, water abstraction or hydro-morphological changes; Dudgeon, 2019; Vörösmarty et al., 2010) had led to alarming rates of biodiversity loss worldwide (Baker et al., 2020; Geist, 2011). In this context, it is fundamental to gain knowledge on the ecological responses to global change-related factors, so that effective management actions could be implemented to protect freshwater resources and biodiversity (Grizzetti et al., 2017), in line with the post-2020 global biodiversity framework (Convention on Biological Diversity, 2021; Nicholson et al., 2021).

Changes in climate and land use and land cover (LULC) are the global change components that most compromise riverine ecosystems worldwide, along with dam construction (Caro et al., 2022; Liermann et al., 2012; Reid et al., 2019). Climate change produces alterations in the historical precipitation and temperature regimes (Bates et al., 2008; Poff et al., 1996), directly affecting water and sediment flows in rivers (Arnell and Gosling, 2013; Schneider et al., 2013; Syvitski, 2003). Moreover, thermal regime changes are critical for riverine biota, causing reductions in species richness, simplification of assemblages, and changes in primary productivity (Durance and Ormerod, 2007; McDonald et al., 2011; Song et al., 2018). LULC changes across the catchment and riparian areas (e.g. deforestation, intensive agriculture, urbanisation) frequently result in habitat degradation (Allan, 2004; Vörösmarty

et al., 2010) and changes in species distribution (Liu et al., 2021; Zeni et al., 2019). At the same time, the hydropower expansion and water supply reservoirs to attend to the increasing demands, lead to flow, thermal, sediment and nutrient regime alteration, loss of habitat connectivity, among other remarkable impacts (Sauer et al., 2010; Vörösmarty et al., 2010; Zarfl et al., 2019). All these pressures cause profound alterations in the temporal and spatial habitat resources and energy fluxes in rivers, producing effects from individual to ecosystem levels, e.g. affecting from organisms' development and life cycles (Larsen et al., 2016) to river primary production (Bernhardt et al., 2022).

In recent years, a growing number of large-scale studies have been investigating the global change-related impacts (i.e. climate change, LULC, and flow alteration) on riverine biodiversity (e.g. Comte et al., 2021; Mouton et al., 2022; Soininen et al., 2016). However, most of them have focused on unique communities or single-species populations, and the use of multiple taxa to assess biodiversity patterns is still rare (but see Pound et al., 2021; Tison-Rosebery et al., 2022), especially in the Iberian Peninsula. Moreover, time-series data from multiple river reaches covering large spatial scales are non-existent or very scarce (Blois et al., 2013). Thus, space-for-time substitution might be an appropriate technique to gain insights into future impacts of global change on river ecosystems (Wogan and Wang, 2018), especially when prediction into future scenarios is avoided (Damgaard, 2019).

Freshwater biological communities (e.g. diatoms, macrophytes, benthic macroinvertebrates, fish) have shown asymmetrical responses to stressors (Guse et al., 2015; Hering et al., 2006), suggesting that the impacts' direction and intensity are highly dependent on food web trophic level or biological and ecological characteristics of the communities under study. Thus, research encompassing large spatial extents, highly variable environmental conditions, and multiple biological communities (e.g. primary producers to apex predators) is paramount to improve the understanding of the effects that global change might have on river ecosystems.

Significant taxonomic variability is expected when carrying out studies covering large environmental gradients (Liu and Wang, 2018), as organisms are filtered according to their ecological requirements (Poff, 1997). However, even across wide environmental gradients, general patterns or trends underlying community assembly are not always evident due to historic, dispersal-related, and evolutionary processes controlling regional species pools (Lessard et al., 2012; Soininen et al., 2016). Thus, integrative approaches to disentangle the influence of these biogeographical processes from the biodiversity-environment relationships are essential (Lessard et al., 2012). In this context, trait-based approaches are a valuable alternative to taxonomy, as they promptly and consistently respond to habitat filters (Mouillot et al., 2013) and

reduce biogeographic constraints imposed by single species at large spatial scales (Bonada et al., 2006; Chen and Olden, 2018; Menezes et al., 2010). Furthermore, traits provide a mechanistic perspective to examine the links between biodiversity and environmental gradients or stressors (Culp et al., 2011; McGill et al., 2006; Olden et al., 2010).

The main goal of this exploratory study is to identify the key dynamic (i.e. climate, LULC, hydrology) and static (i.e. geology, topography) environmental variables influencing the spatial variability of riverine biodiversity. This exercise will serve as a baseline to monitor biological community changes, helping to track anthropogenic effects related to global change on riverine ecosystems. The biological datasets, covering diatom, macroinvertebrate, and fish communities, were originally collected to address the European Water Framework Directive (WFD; European Commission, 2000) water quality objectives in Spain, a country with highly variable environmental conditions (e.g. semiarid, Mediterranean, and temperate climates) in a relatively small area (over 0.5 million km^2). The biological sampling was intentionally limited to streams with good ecological status and free of local anthropogenic pressures, so that community shifts were mainly restricted to large-scale environmental gradients.

In this study, we expect that (1) large-scale environmental gradients (i.e. climate, hydrology, LULC, geology, topography) would have different contributions to structuring diatom, macroinvertebrate, and fish communities, as these organisms have contrasting biological and ecological characteristics. Using exploratory approaches, we expect to (2) identify the main ecological responses associated to climate, LULC, and hydrology (i.e. dynamic variables linked to global change) since these variables can act as filters selecting taxonomic groups and traits vulnerable to anthropogenic stressors. (3) Biological and ecological traits would be useful in unveiling these ecological responses to global change since trait-based variability should be more directly correlated to environmental gradients than taxonomy.

2.2 Methodology

2.2.1 Study area

The study area corresponds to continental Spain (40°23'N, 3°33'E; Figure 2.1). The relief is characterised by an extensive high inland plateau (average of 650 m.a.s.l.) surrounded by mountain ranges, with peaks reaching 3,400 m.a.s.l. (Rivas-Martínez et al., 2004). Different major climatic zones are distributed along the country: mountain climate in high altitudes, with cold winters and abundant precipitations

(annual averages reaching 3,000 mm); oceanic climate in the northwest region; hot steppe climate in the southeast, with minimum rainfall levels in Spain (annual averages <150 mm); and Mediterranean climates with dry and hot summers (AEMET and IMP, 2011; Serrano-Notivoli et al., 2018). The relief-climate interactions generate significant variability in the environmental conditions and hydrological regimes, influencing riverine biodiversity across continental Spain (Peñas et al., 2014).

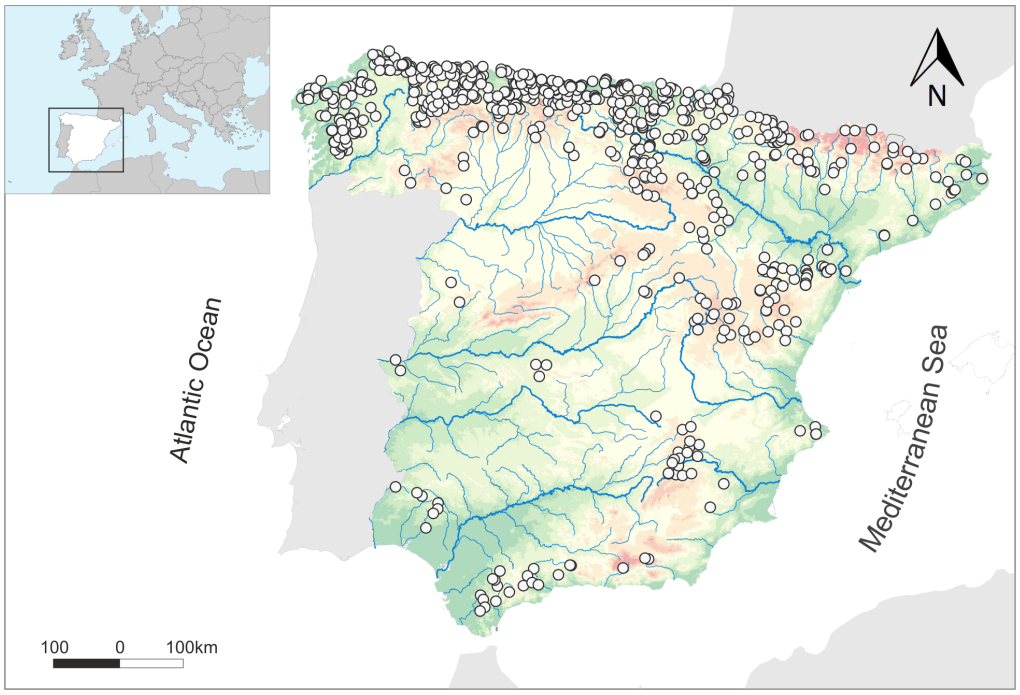


Figure 2.1 Study area (505,990 km^2) and selected sampling sites, in a total of 882 locations (177 for diatoms, 441 for macroinvertebrates, and 264 for fish).

2.2.2 Hydrological and environmental characteristics

To integrate biological, hydrological and environmental information into a spatial framework, we used virtual watersheds (Barquín et al., 2015; Benda et al., 2016) constructed with flow direction inferred from a 10-meter digital elevation model (Peñas and Barquín, 2019). We selected 26 catchment-scale environmental variables (Table 2.1) obtained from national and regional databases and associated to each river network segment. These variables were divided into two groups (see Figure 2.2 and Table 2.1): (1) dynamic environmental variables: climate (average annual temperature, precipitation, and evapotranspiration), LULC (mean fractions in the catchment occupied by agriculture, urbanisation, forests, etc.), and hydrology (non-correlated synthetic hydrological indices); (2) static environmental variables: geology (mean catchment fraction occupied by different geological structures), and topography (slope, altitude, distance to river outlet, and catchment area; i.e. variables

describing the position along the river network). Changes in static variables, for practical purposes, are considered imperceptible over time (e.g. soil type changes occurring over geologic time), whereas dynamic variables (i.e. LULC, climate, hydrology) are expected to go through relevant alterations over shorter timescales, especially when linked to human-driven activities such as climate change or land use change (Stanton et al., 2012).

The hydrological variables were composed of 85 hydrological indices (HI), which were calculated from the normalised daily flow series recorded by 282 natural flow gauges throughout the country provided by the Centre for Hydrological Studies of the Spanish Ministry for Ecological Transition (CEDEX, 2022). The HIs were reduced to a set of non-correlated synthetic indices (SI_{NAT}), and predicted to all river reaches using Random Forest models. The first four SI_{NAT} explained 84% of the total hydrological variance, setting the critical natural flow patterns in Peninsular Spain. They can be individually interpreted according to the HI with the highest contribution (Table A.2 in Appendix A): high $SI_{NAT}1$ scores corresponded to low intra-annual variability of daily flows; high $SI_{NAT}2$ scores were associated to longer but less frequent high flow events; high $SI_{NAT}3$ scores corresponded to low interannual variability of the minimum flow timing; high $SI_{NAT}4$ scores were associated to increased magnitude and variability of April flows, i.e. higher and variable spring flows (Peñas and Barquín, 2019).

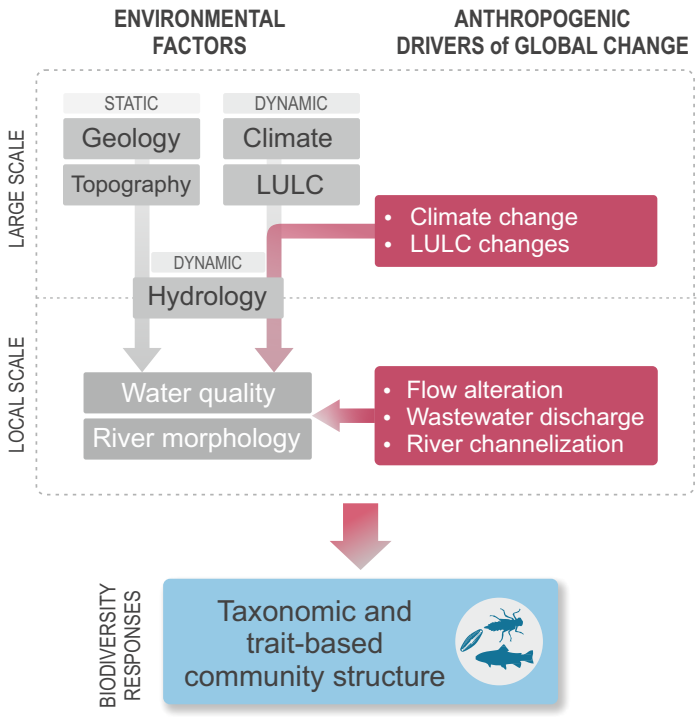


Figure 2.2 Interactions among environmental factors at large- and local-spatial scales (grey boxes), anthropogenic pressures (red boxes), and riverine biological communities.

Table 2.1 Catchment-scale environmental variables describing dynamic (climate, LULC, and hydrology) and static (topography and geology) variables in diatoms, macroinvertebrates, and fish sampling sites. Values refer to the mean ± sd catchment values upstream of the sampling site.

Code	Description	Diatoms	Macroinvertebrates	Fish
		(n=441)	(n=177)	(n=264)
TEMP	Mean annual temperature (°C)	11.9 ± 2.6	11.6 ± 2.3	11.8 ± 2.3
PREC	Mean annual precipitation (mm)	864.3 ± 327.4	1162.4 ± 354.3	1132.1 ± 343
EVT	Mean annual evapotranspiration (mm)	477.2 ± 66.1	511.9 ± 77.5	511.4 ± 75.5
URB	Fraction occupied by urbanisation	0 ± 0	0 ± 0	0 ± 0
AGR	Agriculture	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.1
PAS	Pastures	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.1
BLF	Broadleaf forests	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2
CNF	Coniferous forests	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2
PLT	Plantations	0 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
SSH	Heathlands and shrubs	0.2 ± 0.2	0.3 ± 0.2	0.3 ± 0.2
DEN	Denuded areas	0 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
$SI_{NAT}1$	Non-correlated	-1.49 ± 0.62	0.15 ± 0.39	0.10 ± 0.45
$SI_{NAT}2$	synthetic	0.21 ± 0.71	-0.31 ± 0.62	-0.30 ± 0.67
$SI_{NAT}3$	hydrological	-0.04 ± 0.49	-0.09 ± 0.5	-0.14 ± 0.48
$SI_{NAT}4$	indices	0.24 ± 0.39	-0.03 ±0.45	-0.07 ± 0.42
AREA	Catchment size (km^2)	226 ± 502.2	158.6 ± 330.6	225.6 ± 410.1
OUT	Relative distance to river outlet (0 to 1)	0.5 ± 0.3	0.4 ± 0.3	0.3 ± 0.3
ELEV	Elevation above sea level (m)	653.1 ± 351.7	447.6 ± 358.9	372.8 ± 339
SLP	Slope in segment (0-1)	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.2
CALC	Fraction occupied by calcareous rocks	0.5 ± 0.4	0.3 ± 0.4	0.4 ± 0.4
CONG	Conglomerate rocks	0.3 ± 0.3	0.2 ± 0.3	0.2 ± 0.
SAND	Sandstone	0 ± 0.1	0.1 ± 0.2	0.1 ± 0.2
SDIM	Sedimentary rocks	0 ± 0.1	0 ± 0.1	0 ± 0
SLIC	Siliceous rocks	0.1 ± 0.3	0.2 ± 0.3	0.2 ± 0.3
SLTE	Slates	0 ± 0.1	0 ± 0.1	0 ± 0.2
VOLC	Volcanic rocks	0 ± 0.1	0.1 ± 0.3	0.1 ± 0.3

2.2.3 Characterisation of biological communities and traits

In this study, the national biological monitoring databases (NABIA; Spanish Royal Decree 817/2015 on Water Policy; BOE, 2005) were used. These databases have been compiled since 2008 by the Spanish Ministry for the Ecological Transition (MITECO), gathering biological surveys carried out at the national level to assess water bodies' ecological status in compliance with the European WFD (European Commission, 2000).

From these databases, only biological community samples collected in wadeable streams between June and October were selected to reduce the intra-annual variability (Leathwick et al., 2005). Then, to reduce the effects of local anthropogenic pressures commonly found in rivers (see red boxes in Figure 2.2), we retained only samples surveyed in river reaches catalogued as "good" or higher ecological and chemical status (River Basin Management Plans 2015-2021; BOE, 2005) and unaffected by local anthropogenic pressures such as dams, embankments, or significant water abstractions upstream. This site selection, covering only minimally disturbed streams, allowed looking into the effects of dynamic variables over the selected riverine biological communities without the confounding effect of other stressors, such as water pollution, hydromorphological pressures or the presence of dams and reservoirs (see Figure 2.2).

A total of 177 diatom, 441 macroinvertebrate, and 264 fish sampling sites were finally retained (Figure 2.1). Physico-chemical measurements were not available in all sampling sites and therefore were not included in the study.

Diatoms and fish were reported at the species level. Macroinvertebrates were mostly identified at the family level, as required by the IBMWP score system (Alba-Tercedor et al., 2002), except for Acariformes and Oligochaeta. Ostracods were removed from the taxa list as they are considered microcrustaceans and not accounted for in all surveys. In sites sampled for multiple years, the abundance of taxa was averaged to obtain a site-specific assemblage structure, following Paavola et al. (2003) and Filker et al. (2016). Given the large number of taxa retained, rare taxa in diatom and macroinvertebrate communities were eliminated (those representing less than 2% of total sampled individuals when considering the total number of occurrences; Lavoie et al., 2009).

Diatom species were assigned to ecological guilds (high and low profile, planktonic, motile), life forms (colonial, stalked, adnate, pioneer), and cell sizes (biovolume; Table B.1 in Appendix B). Macroinvertebrate families were assigned to biological traits using a fuzzy-coding approach, describing feeding habits, food types, maximum body size, life span, the annual number of reproductive cycles, aquatic stages, modes of respiration, locomotion, reproduction, dissemination, and resistance forms (see

Table B.2 in Appendix B). Since macroinvertebrate traits were mainly reported at the genus level (Tachet et al., 2010), each trait affinity was averaged and re-scaled to the family level using affinities of all genera computed within a family. Although the use of family-level identification can result in loss of ecological information, previous studies (Dolédec et al., 2000; García-Roger et al., 2014; Gayraud et al., 2003; Gutiérrez-Cánovas et al., 2019) found it had minor impacts on the trait-based description of macroinvertebrate communities when compared to genus level. Last, fish species were assigned to traits describing maximum body length, overall tolerance to stressors, habitat use, feeding habits and habitat, and migration patterns based on available information for Iberian species (Table B.3 in Appendix B).

2.2.4 Data analysis

We explored the relationships among environmental variables and biological community spatial patterns using two different methods. Firstly, using redundancy analysis (RDA; Legendre and Legendre, 2012), we identified the environmental factors that best explained the communities' spatial variability. The RDA establishes a model measuring the variance of the biological datasets in relation to the environmental characteristics (Legendre and Legendre, 2012). For each biotic group, using both taxonomic and trait-based datasets (i.e. community-weighted mean trait values; CWM), a subset of explanatory variables was obtained using a stepwise forward selection procedure with 9999 permutations and a double-stopping criterion (α<0.05 and the global model adjusted coefficient of determination) to avoid type I error inflation (Blanchet et al., 2008). The CWM trait approach calculates an aggregated trait value based on the relative abundance of species in a given community (Ackerly and Cornwell, 2007). Then, the adjusted coefficient of determination (R^2_{adj}) of each RDA model was computed, accounting for the number of predictors and samples, which allowed comparisons between groups (Peres-Neto et al., 2006). For taxonomy matrices, Hellinger transformation was used to reduce the influence of abundant taxa in the community structure (Legendre and Gallagher, 2001); for trait matrices, the average trait expression of all Hellinger-transformed taxa was weighted by taxa abundances in each site (Kleyer et al., 2012).

The second analysis investigated the overall and individual trait-environmental relationships using RLQ and fourth-corner analyses (Dray et al., 2014). These two methods constitute a powerful approach to investigate trait-environment relationships in community ecology (Peres-Neto et al., 2017), with numerous examples in the literature (e.g. Brown et al., 2019; Wu et al., 2019b; Zeni et al., 2019). The RLQ analysis (Dolédec et al., 1996), an exploratory ordination method, combines three

tables: table R ($n \times m$), containing the measurement of m environmental variables at n sites; table L ($n \times p$), with the abundance of p taxa at n sites; table Q ($p \times s$), describing s traits related to the same p taxa. The method generates a fourth matrix representing the cross-variance structure explained by environmental variables and functional traits and provides a broad overview of how traits and environmental characteristics are associated (Brown et al., 2014; Thioulouse et al., 2018). The overall significance of the trait-environment relationship can be assessed with 9999 permutations using a two-step procedure (Model 6; Dray et al., 2014), which simultaneously tests the permutation of sites and species while controlling the type I error (ter Braak et al., 2012). The fourth-corner method, in contrast, computes a matrix ($s \times m$) with the one-to-one trait-environment correlations (Dray et al., 2014). The combination of these two methods allowed us to identify the main patterns and individual trait-environment relationships. In addition, we evaluated the responses of individual taxa to environmental variables using Spearman Rank Correlations in the three biotic groups.

Prior to the analyses, the environmental variables were standardised (zero mean and unit standard deviation) to reduce the effects of different measurement units and improve normality (Quinn and Keough, 2002). All analyses were performed in the R environment (R Core Team, 2020) with the `ade4` (Dray and Dufour, 2007), `adespatial` (Dray et al., 2020), and `vegan` (Oksanen et al., 2019) packages.

2.3 Results

2.3.1 Contribution of environmental variables to the biological variability

The selected environmental variables obtained with the RDA analyses (Table 2.2 and Table S2.1 in Supplementary Material) accounted for 14.9%, 19.0%, and 35.9% of the explained taxonomic variation of diatom, macroinvertebrate, and fish communities, respectively. Dynamic variables (i.e. linked to climate, LULC, and hydrology) showed significant contributions in the three biotic groups. Climate-related variables were clearly the most important ones explaining the taxonomic variation in diatoms (TEMP accounting for 31% of the total explained variability, see Table S2.1 in Supplementary Material), followed by hydrology and LULC. In macroinvertebrate communities, climate also played a major role in explaining spatial variability (PREC and TEMP accounting for 42% of the total explained variability), followed by LULC. In fish communities, hydrology had a major contribution ($SI_{NAT}2$ accounted for 31%

of the total explained variability), followed by climate and LULC. Among the static variables, topography (SLP, ELEV) and geology (SLIC, CALC), also had significant contributions in the three biotic groups, especially in macroinvertebrates.

Table 2.2 Subset of environmental variables composing RDA models explaining taxonomic and trait-based diatom, macroinvertebrate, and fish communities' variability. The adjusted coefficient of determination (R^2_{adj}) values are shown. Variables were selected with a forward selection procedure (see Table 2.1 for variable codes). The contribution of each independent variable is shown in Table S2.1 in Supplementary Material.

	Biotic group	**Selected variables**	R^2_{adj}
Taxonomy	Diatoms	TEMP, SLP, SLIC, ELEV, $SI_{NAT}4$, $SI_{NAT}1$, $SI_{NAT}3$, CNF, PAS, PLT, OUT, SDIM	0.149
	Macroinvertebrates	PREC, TEMP, ELEV, SLP, CALC, $SI_{NAT}1$, CONG, AGR, PAS, VLC, URB, DEN, SLTE, AREA, CNF, SAND	0.190
	Fish	$SI_{NAT}2$, CALC, ELEV, CONG, $SI_{NAT}1$, AREA, SSH, $SI_{NAT}4$, SLP, PREC,TEMP, BLF, OUT, CNF, URB, $SI_{NAT}3$, AGR, VLC, PLT, EVT	0.359
Traits	Diatoms	AGR, BLF, SDIM, URB	0.129
	Macroinvertebrates	CALC, AGR, ELEV, PREC, $SI_{NAT}1$, PAS, SLP, VOLC, URB, CNF, AREA, SLIC, TEMP	0.192
	Fish	PREC, SSH, $SI_{NAT}1$, CNF, CALC, AGR, CONG, TEMP, OUT, PLT, $SI_{NAT}3$, AREA	0.546

When using community-weighted mean traits, the explanatory variables selected by the CWM-RDA (Table 2.2 and Table S2.1 in Supplementary Material) accounted for 12.9%, 19.2%, and 54.6% of the diatom, macroinvertebrate, and fish communities' variability, respectively. LULC-related variables (AGR, BLF, URB) accounted for more than 80% of the explained variation in diatom traits variability (see Table S2.1 in Supplementary Material). In contrast, calcareous bedrocks (CALC) and altitude (ELEV), both static environmental variables, together with agriculture (AGR) explained 58% of the macroinvertebrate trait variability. Fish trait variability was mainly related to precipitation (PREC; 44% of the explained variation), followed

by shrub and heathlands (SSH) and the intra-annual variability of daily flows ($SI_{NAT}1$).

In both taxonomic and trait-based analyses, fish communities had the highest amount of variation explained by the catchment-scale environmental variables (on average, 45.3%, in contrast to 13.9% for diatoms and 19.1% for macroinvertebrates). Moreover, when using the trait-based approach, the environmental variables accounted for larger amounts of explained variation in fish (an increase of 19% in relation to taxonomy), whereas in diatom and macroinvertebrate communities, the contribution of environmental variables hardly changed (decrease of 2% and an increase of <1%, respectively in relation to taxonomy).

2.3.2 Diatoms' multidimensional analysis

In the 177 diatom samples, 95 diatom species were retained. The most abundant genera were *Achnanthidium* (corresponding to 42.2% of all sampled diatoms), *Cocconeis* (14.8%), *Gomphonema* (9.4%), and *Nitzschia* (5.4%). *Achnanthidium minutissimum* was the most ubiquitous species, with an average relative abundance of 20.1% in all sampling sites. The high-profile guild, stalked life forms, and small-sized diatoms dominated most locations.

The first two axes of the RLQ ordination representing diatom assemblages (Figure 2.3) accounted for 78.2% of the cross-variance between environmental variables and diatoms' traits. A global RLQ permutation test indicated a non-significant overall association between environment and traits (permutation across sites $p<0.001$; permutation across species $p=0.106$).

The first axis (64.0% of the cross-variance) showed a marked gradient of stream size associated to topography, climate, LULC, and hydrology. Mountain and headwater streams, characterised by high slopes (+SLP) and precipitation intensities (+PREC), were positively correlated to species such as *Achnanthidium pyrenaicum* (SLP r=0.26, PREC r=0.26; $p<0.001$ in all cases) and *Gomphonema pumilum* (SLP r=0.15, PREC r=0.21; $p<0.04$). These sites were segregated from lowland streams, which presented larger catchment areas (+AREA), agricultural (+AGR) and urban activities (+URB), and longer but infrequent high flow events (+$SI_{NAT}2$), and were positively linked to species such as *Amphora pediculus* (AREA r=0.32, AGR r=0.46, URB r=0.44, $SI_{NAT}2$ r=0.19; $p<0.01$). This gradient was also reflected in the diatom traits, with higher dominance of stalked diatoms (Stalk, e.g. *Achnanthidium lineare*) in headwater streams, which were replaced by adnate life forms (e.g. *Cocconeis euglypta*), pioneer (e.g. *A. pediculus*), and motile diatoms (e.g. *Nitzschia fonticola*) downstream (see the fourth-corner analysis in Figure S2.1 in Supplementary Material).

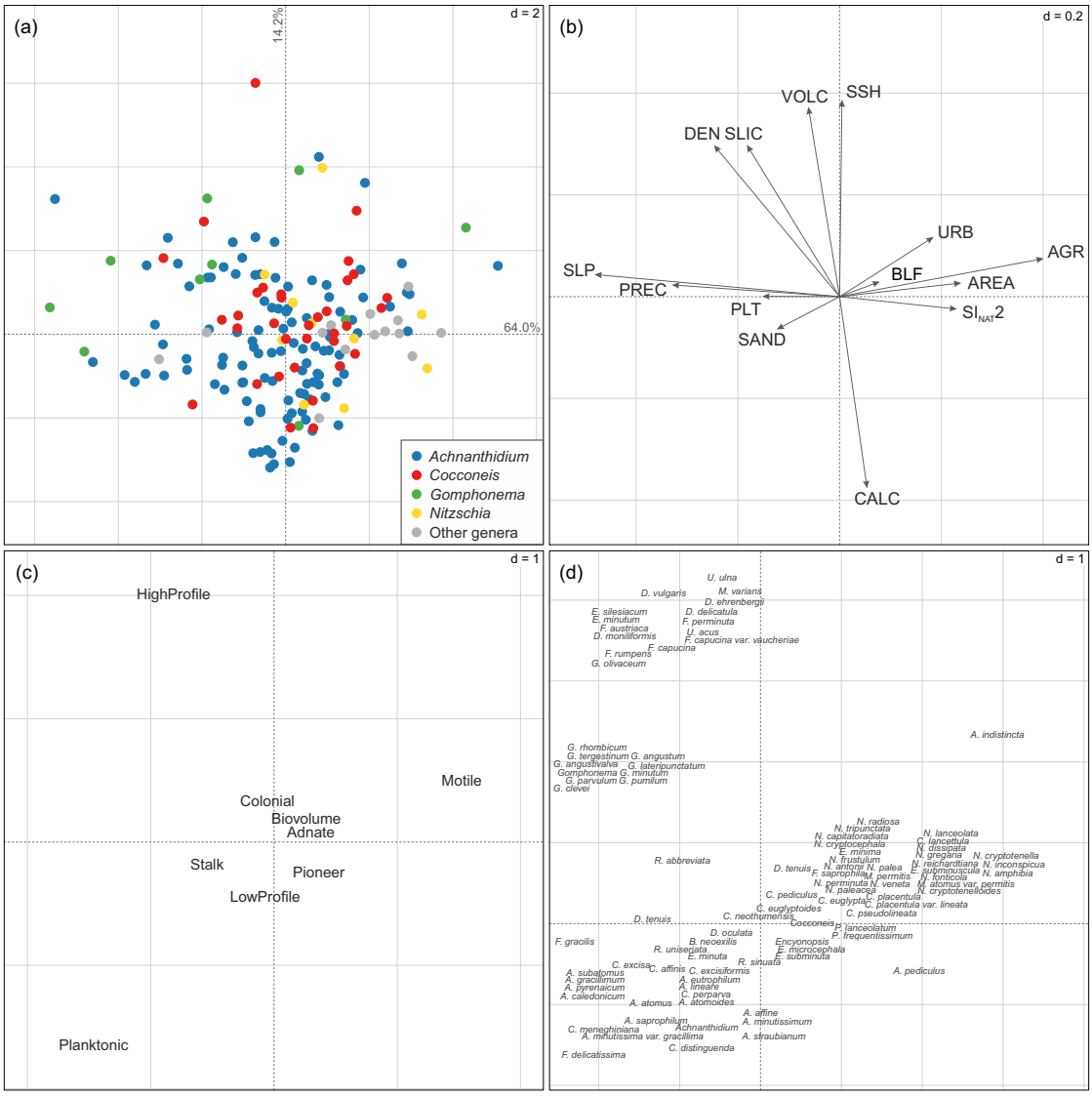


Figure 2.3 Ordinations showing the first two RLQ axes on diatoms communities. (a) Site scores according to the diatoms genus dominance; (b) Coefficients for key environmental variables (see Table 2.1 for codes) with the strongest links to traits (see Figure S2.1 in Supplementary Material). (c) Coefficients for functional traits (see Table B.1 for codes). (d) Species scores. The "d" values give the grid size for scale comparison across the graphs.

The second axis (14.2%) was marked by a gradient related to lithology and LULC. Sites with volcanic (+VOLC) and siliceous (+SLIC) geology, shrubs and heathlands (+SSH), and denuded areas (+DEN) were positively correlated with the diatom species *Planothidium frequentissimum* (SSH r=0.15, VOLC r=0.17; p<0.04), as well as high profile diatoms (HighProfile, e.g. *Gomphonema minutum*). These sites were replaced by karstic streams (+CALC), which were significantly correlated to species such as *A. pyrenaicum* (r=0.29, p<0.001) and *Cymbella excisa* (r=0.26, p<0.001) and low profile (LowProfile, e.g. *A. minutissimum*) and planktonic (e.g. *Fragilaria delicatissima*) guilds.

2.3.3 Macroinvertebrate' multidimensional analysis

Sixty macroinvertebrate taxa were retained. The four most abundant families covered 51.8% of all identified macroinvertebrate individuals: Chironomidae (19.3%), Baetidae (15.8%), Elmidae (8.5%), and Gammaridae (8.3%).

The first two axes of the RLQ ordination (Figure 2.4) accounted for 81.6% of the cross-variance between environmental variables and macroinvertebrate traits. A global RLQ permutation test did not show a significant overall trait-environment association (permutation across sites $p<0.001$; permutation across species $p=0.763$).

Similar to the diatom ordination, the first RLQ axis (44.4%) represented a gradient of stream size associated to climate, lithology, and hydrology. High precipitation (+PREC), siliceous geology (+SLIC), and shrubs and heathlands (+SSH), i.e. headwater streams susceptible to flow disturbances, were correlated to EPT taxa such as Nemouridae (PREC r=0.37; SLIC r=0.20; SSH r=0.24; $p<0.001$ in all cases) and Sericostomatidae (PREC r=0.28; SLIC r=0.29; SSH $p=0.20$; $p<0.001$ in all cases). In contrast, sites with calcareous geology (+CALC), higher presence of agriculture (+AGR) and pastures (+PAS), long but infrequent high flow events (+$SI_{NAT}2$), i.e. karstic and lowland rivers with stable flow regimes, were highly correlated to Gammaridae (CALC r=0.34; AGR r=0.16; PAS r=0.162; $p<0.001$ in all cases) and Hydrobiidae (CALC r=0.25; AGR $p=0.27$; PAS r=0.133; $p<0.005$ in all cases). This gradient was also reflected in macroinvertebrate traits. Siliceous (+SLIC) and steeper (+SLP) streams were associated to organisms with aquatic passive dissemination (AquPassive, e.g. Dixidae larvae), aquatic eggs (Egg, e.g. Lymnaeidae, Heptageniidae), and macrophytes as a food source (Macrophytes, e.g. Ancylidae, Bithyniidae). They were replaced by organisms with no resistance strategies (NoResistance, e.g. Dytiscidae, Glossiphoniidae), with terrestrial egg clutches (ClutchTerr, e.g. Athericidae, Hydraenidae), and piercers (Piercer, e.g. Athericidae, Gerridae; see also the fourth-corner analysis in Figure S2.2 in Supplementary Material) in karstic and stable lowland streams.

Additionally, a mountain-lowland stream gradient related to climate, topography, and LULC and partly linked to the second axis (37.2%) marked the segregation of non-insect dominances. Higher annual temperatures (+TEMP) and agricultural areas (+AGR), i.e. lowland rivers, were correlated to Gammaridae (TEMP r=0.16, $p=0.001$; SLP r= -0.25, $p<0.001$) and Hydrobiidae (TEMP r=0.26, $p<0.001$; SLP r=-0.21, $p<0.001$), which were the main representative taxa of non-insects. In contrast, steep areas (+SLP) with low temperatures (-TEMP) and low agricultural activity (-AGR) were correlated to EPT taxa such as Heptageniidae (TEMP r=-0.39; AGR r=-0.32; SLP r=0.40; $p<0.001$ in all cases). This gradient was also evident

in macroinvertebrate traits, with lower mean temperatures in headwater streams favouring scrapers (e.g. Heptageniidae, Lymnaeidae), whereas lowland rivers with higher mean temperatures and agricultural activities were associated to organisms with maximum body sizes between 0.25-0.5cm (Small, e.g. Haliplidae, Hydraenidae), no resistance strategies, and piercers.

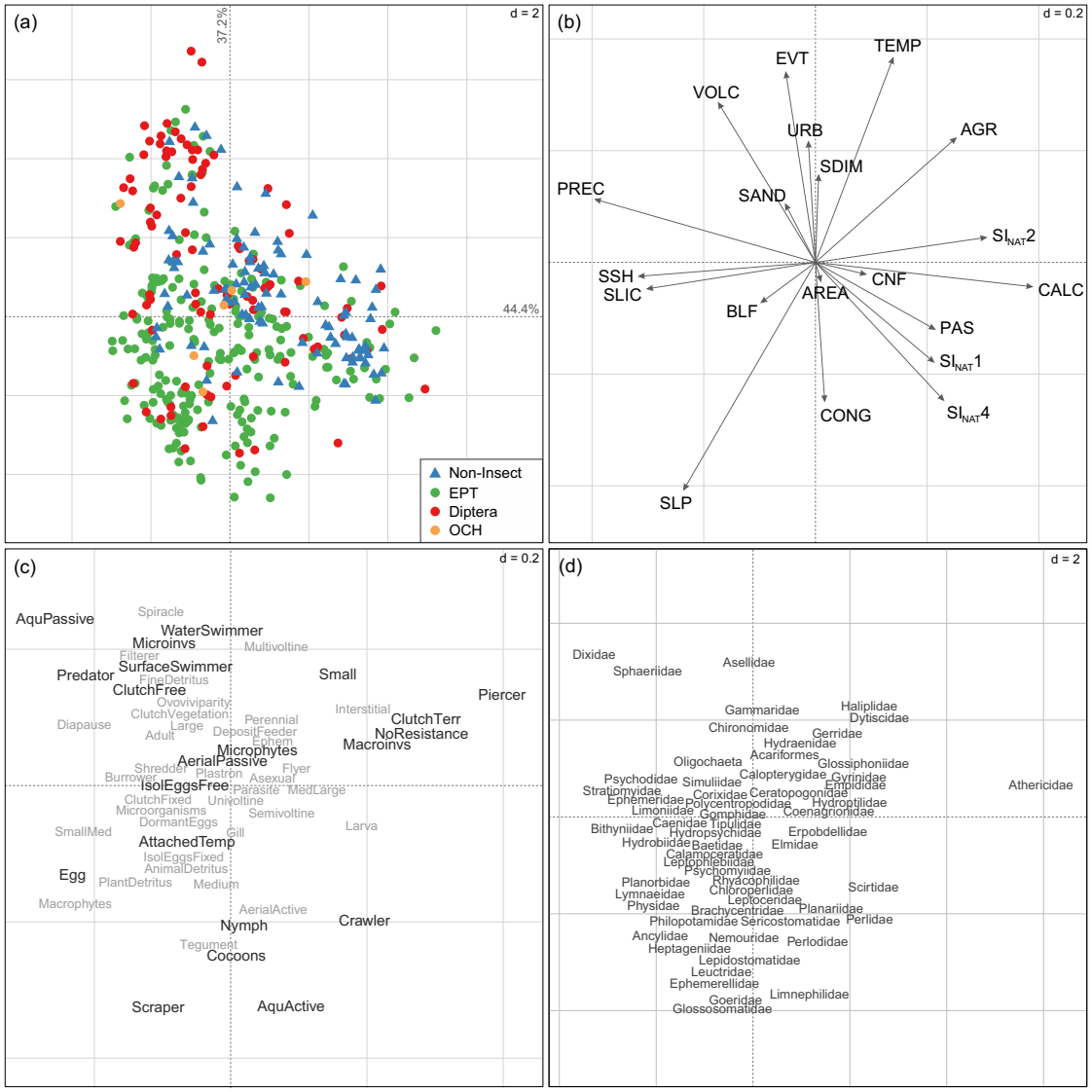


Figure 2.4 Ordinations showing the first two RLQ axes on macroinvertebrate communities. (a) Site scores according to the macroinvertebrate taxa dominance. (b) Coefficients for key environmental variables (see Table 2.1 for codes) with strongest links to traits (see Figure S2.2 in Supplementary Material); (c) coefficients for functional traits (see Table B.2 for codes). Traits with non-significant relationships are represented in grey (Figure S2.2). (d) Taxa scores. The "d" values give the grid size for scale comparison across the graphs.

2.3.4 Fish' multidimensional analysis

Thirty-eight species of fish were retained for the community analyses. The most abundant taxa were *Salmo trutta* (corresponding to 29.8% of all sampled fish and present in most samples), *Phoxinus bigerri* (28.7%), and *Parachondrostoma miegii* (6.7%).

The first two axes of the RLQ ordination (Figure 2.5) accounted for 86.2% of the cross-variance between environment and fish traits. A global RLQ permutation test indicated a significant trait-environment relationship (permutation across sites $p<0.001$; permutation across species $p=0.041$).

Along the first axis (72.6%), a major gradient of stream size related to climate, hydrology, and LULC segregated sites dominated by salmonids from other subfamilies' dominances. Large catchments (+AREA), high magnitude and variability of spring flows (+$SI_{NAT}4$), and coniferous forests (+CNF), i.e. lowland rivers, were significantly correlated to species such as *Luciobarbus graellsii* (AREA r=0.36; $SI_{NAT}4$ r=0.14, CNF r=0.27; $p<0.02$ in all cases) and *P. miegii* (AREA r=0.32, CNF r=0.27; $p<0.001$ in all cases), as well as non-migratory fish (NonMigratory, e.g. *P. bigerri*). In contrast, sites with high precipitation (+PREC), shrubs and heathlands (+SSH), and siliceous geology (+SLIC), i.e. mountain rivers, were significantly correlated to species such as *S. salar* (PREC r=0.27; SSH r=0.21; SLIC r=0.32; $p<0.001$ in all cases) and *S. trutta* (PREC r=0.48; SSH r=0.29; SLIC r=0.36; $p<0.001$ in all cases) and favoured long-distance migration (LongMigration, e.g. *Salmo salar*) and fish inhabiting the water column (WaterColumn, e.g. *P. bigerri*; see also the fourth-corner analysis in Figure S2.3 in Supplementary Material).

Additionally, a mountain-lowland stream gradient, partly linked to the second axis (13.6%), was associated to hydrology, LULC, and topography and segregated sites dominated by Barbinae from other subfamilies. Long but infrequent high flow events (+$SI_{NAT}2$) and agricultural areas (+AGR), i.e. lowland rivers with more stable flow regimes, were positively correlated to *Luciobarbus sclateri* ($SI_{NAT}2$ r=0.26, $p<0.001$) and *Gobio lozanoi* ($SI_{NAT}2$ r=0.17, $p=0.007$; AGR r=0.18, $p=0.003$), whereas steeper areas (+SLP) were positively correlated to *S. trutta* (r=0.25, $p<0.001$) and *S. salar* (r=0.23, $p<0.001$). Regarding traits, lowland rivers were associated to non-migratory species (NonMigratory, e.g. *P. bigerri*, *Squalius carolitertii*), while mountain streams were related to fish performing long migration movements (LongMigration), rheophilic species (Rheophilic, e.g. *P. miegii*), and large body sizes (MaxLength, e.g. *A. anguilla*).

Another environmental gradient associated to hydrology, lithology, and LULC contributed to fish community variability. Calcareous rocks (+CALC) and pastures

(+PAS) were positively correlated to *L. graellsii* (CALC r=0.36, PAS r=0.27; p<0.001), *P. bigerri* (CALC r=0.36, PAS r=0.31; p<0.001), *Barbatula barbatula* (CALC r=0.23, PAS r=0.18; p<0.003). In contrast, low interannual variability of the minimum flow timing (+$SI_{NAT}3$) was significantly correlated to *Achondrostoma arcasii* (r=0.20, p=0.001) and intolerant species (Intolerant, e.g. *Alosa alosa*, *S. salar*).

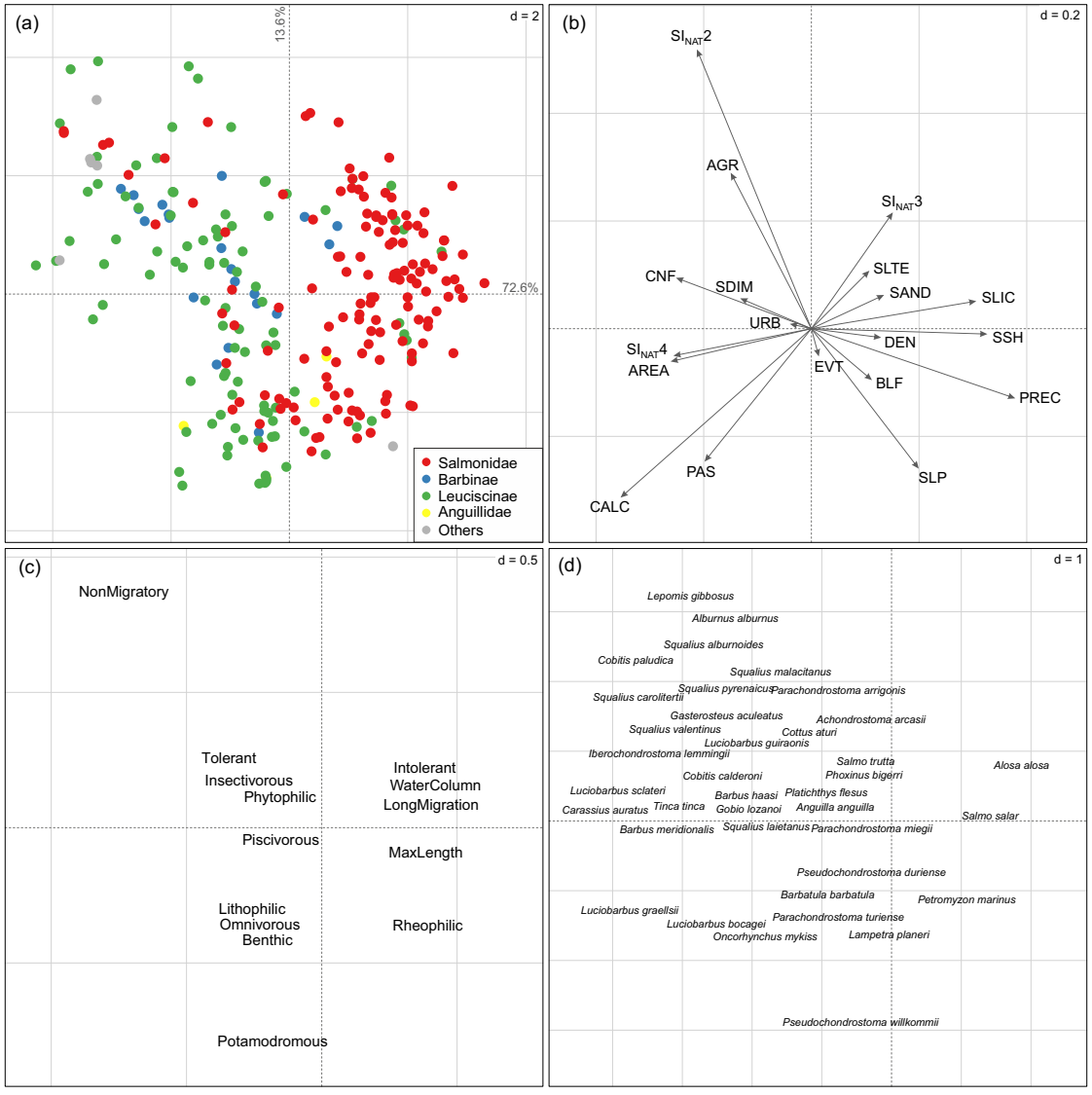


Figure 2.5 Ordinations showing the first two RLQ axes on fish communities. (a) Site scores according to the fish subfamily dominance. (b) Coefficients for key environmental variables (see Table 2.1 for codes) with strongest links to traits (see Figure S2.3 in Supplementary Material). (c) Coefficients for functional traits (see Table B.3 for codes). (d) Species scores. The "d" values give the grid size for scale comparison across the graphs.

2.4 Discussion

In this study, the major environmental gradients related to the spatial variability of diatom, macroinvertebrate, and fish communities were investigated. As expected, the power of the landscape-scale environmental variables contributing to the biological communities' variability differed among biotic groups, taxonomic, and trait-based approaches. Both dynamic (temperature, precipitation, extreme flow events, agricultural and urban areas) and static (slope, altitude, calcareous and siliceous geology) variables contributed significantly to the observed biological variability and outlined environmental gradients of community change. Although these approaches are not valid to demonstrate specific cause-effect relationships, the results are highly useful to propose specific hypotheses and future research directions regarding the potential effects of global change on riverine ecosystems.

2.4.1 The role of dynamic environmental factors

Climate, LULC, and hydrology showed significant contributions to the three biotic groups' spatial variability, suggesting these communities might be highly sensitive to global change effects (see Table 2.3).

Our findings showed a significant influence of hydrology and climate on diatom communities' spatial variability, in agreement with previous studies (Guo et al., 2020; Wu et al., 2019b). Pioneer diatoms, *Nitzschia*, and other genera dominances (e.g. *Amphora*, *Navicula*) were positively linked to longer but less frequent high flow events but negatively linked to precipitation. This pattern is expected since loosely attached diatoms (e.g. *Nitzschia* and *Navicula*) are more susceptible to drift and are easily displaced from the periphyton after floods (Biggs and Thomsen, 1995; Passy, 2007; Schneck and Melo, 2012). Moreover, mountain streams (i.e. steep headwaters, where agriculture or urbanisation are usually less intense than in lower areas) were associated to high profile and stalked diatoms, whereas lowland rivers with more intensive human land uses were linked to motile diatoms (see also Table 2.3). The lower grazing pressures usually encountered in headwaters may explain the dominance of diatoms growing in the biofilm upper layers (e.g. stalked and high profile; Marcel et al., 2017; Steinman, 1996). The motile guild increased along nutrient gradients: *Nitzschia* (a genus typical of nutrient-rich and organically polluted waters; Biggs and Smith, 2002; Passy, 2007) and other genera dominances (e.g. *Amphora*, *Navicula*) were replaced by the *Achnanthidium* genus, which is adapted to low nutrient conditions (Berthon et al., 2011; Rimet and Bouchez, 2012).

Hydrology played a significant role in the macroinvertebrate community spatial variability. The presence of macroinvertebrates with active dissemination modes (aquatic or aerial) was associated to the increased magnitude and variability of spring flows. Spring flow disturbances are known to be crucial for univoltine and seasonal migrating insects (e.g. Coleoptera), as these disturbances may occur during a sensitive part of their life cycle (Lytle, 2008; Williams, 1996). Moreover, climate and LULC influenced macroinvertebrates' spatial variability, as increases in the mean temperature, urban, and agricultural land uses favoured small-sized, perennial, and multivoltine macroinvertebrates, while the relative abundance of scrapers (e.g. some mayflies and caddisflies) decreased. Temperature, daylight duration, and other climatic factors are known for controlling the development, life history, spawning times, and distribution of macroinvertebrates and other organisms at the catchment and larger spatial scales (Lancaster and Downes, 2013). Therefore, climate change can cause profound changes in macroinvertebrate communities (Durance and Ormerod, 2007; Flenner et al., 2010), as well as human land-use intensification and habitat loss, which are also major drivers of biodiversity losses in rivers (Caro et al., 2022; Pereira et al., 2012). In line with these results, Mouchet et al. (2010) reported increases in the populations of multivoltine species and longer adult life spans, which were linked to climate and land use changes during a 26-year period in New Zealand. Other studies, in agreement with our findings, also showed that global warming had a significant impact on macroinvertebrates, with increasing water temperatures leading to the homogenisation of EPT assemblages (Timoner et al., 2020) and the predominance of resistance and resilience traits (small-sized organisms, fast and seasonal development) in streams affected by urban and agricultural activities (Liu et al., 2021).

Fish assemblages also demonstrated important responses to dynamic variables. Decreases in precipitation and frequency of high flow events seemed to favour generalists (e.g. tolerant, non-migratory) over specialists (e.g. migratory species), as also found elsewhere (Freitas et al., 2013; Whiterod et al., 2015; Zampatti et al., 2010). Drought events and changes in hydrological patterns can be especially harmful to migrant species, as their movements may be constrained by the loss of connectivity among habitats (Freitas et al., 2013; Milton, 2009).

2.4.2 The role of static environmental drivers and interactions among environmental variables

Static variables, in combination with dynamic variables, played a key role in the main community shifts identified in this study. Topography (slope and catchment

Table 2.3 Main ecological responses to dynamic (i.e. global change-related) variables identified in diatoms, macroinvertebrates, and fish assemblages (increase and decrease in abundance of taxonomic and functional groups) in continental Spain.

Decrease in precipitation levels and increase in temperature	**Decrease of natural forest by agriculture, urbanisation, or others**	**Increase in the number of high flow events and reduction in their length**
- Increase of pioneer diatoms (e.g. *A. pediculus*)	- Increase of motile diatoms (e.g. *Nitzschia fonticola*)	- Increase of rheophilic fish (e.g. *Parachondrostoma turiense*)
- Increase of non-migratory fish species (e.g. *P. bigerri*)	- Increase of small-sized macroinvertebrates, with no resistance strategies	
	- Increase of non-migratory fish species (e.g. *P. bigerri*)	
- Decrease of scrapers (e.g. mayflies and caddisflies)	- Decrease of stalked diatoms (e.g. *Cymbella excisa*, *Rhoicosphenia abbreviata*)	- Decrease of pioneer diatoms (e.g. *A. pediculus*)
	- Decrease of macroinvertebrates with active aquatic dissemination (e.g. Nemouridae, Ephemerellidae)	- Decrease of piercers (e.g. Athericidae, Gerridae)
	- Decrease of intolerant fish species (e.g. *Alosa alosa*)	

area as surrogates for the position along the river network), climate (precipitation and temperature), LULC (agricultural and urban land uses), and hydrology (magnitude and frequency of high flow events) were related to community changes across the upstream-downstream gradient, such as the replacement of stalked diatoms, EPT dominances, and long-distance migratory fish in headwaters by pioneer and motile diatoms, non-insects, and non-migratory fish in lowland streams. Previous research reported similar findings, with temperature and position along the river network as the major factors controlling the distribution of diatom (Potapova and Charles, 2002), macroinvertebrates (Griffith et al., 2001; Verdonschot, 2006), and fish communities (Buisson and Grenouillet, 2009; Troia and McManamay, 2019). In the ongoing global change context, this altitudinal gradient emphasises the vulnerability of riverine biodiversity. Temperature increases and land use changes (e.g. forest loss) accelerate the upslope movement of species to seek suitable habitats (Guo et al., 2018); at the

same time, the rate of warming is enhanced with altitude, increasing pressure on mountain ecosystems (Pepin et al., 2015).

Our findings also highlight that dynamic variables such as temperature, precipitation, and agricultural land use are often correlated in strong hierarchical relationships with natural static (e.g. altitude, latitude, position along the river network, geomorphology; McCain and Grytnes, 2010; Vannote et al., 1980) and anthropogenic (e.g. land uses; Allan, 2004) gradients, what could lead to an overestimation of variables' contribution (Snelder and Lamouroux, 2010). Thus, the results emphasise the necessity of considering static and dynamic variables, as well as their interactions, when assessing global change impacts.

2.4.3 Study and method limitations

As expected, landscape-scale variables had different contributions to the diatom, macroinvertebrate, and fish communities' variability. Relatively low amounts of explained variation and non-significant overall environmental-trait relationships were observed in diatoms and macroinvertebrates. These results could be attributed to the lack of relevant local-scale environmental descriptors (e.g. water quality, physical habitat characteristics at the reach level, etc.), which were not available in all sampling sites and hence not included in the analyses. Although the study focused on large-scale environmental gradients, these local-scale descriptors are known for controlling diatom and macroinvertebrate community structure and composition (e.g. Álvarez-Cabria et al., 2017; Dong et al., 2012; Soininen et al., 2016). In contrast, when considering fish assemblages, our environmental predictors explained relatively higher amounts of biological variation and the global relationship between traits and environmental variables was significant. These findings suggest that landscape-scale environmental variables have an important influence on fish taxonomic and trait-based spatial variability, as also seen elsewhere (Esselman and Allan, 2010).

The noisiness of macroinvertebrate datasets, which were mainly reported at the family level, could also reflect on their low explained variation. Although the macroinvertebrate family-level taxonomy is considered suitable for cost-effective water quality assessment (Alba-Tercedor et al., 2002; Chessman et al., 2007), it can mask some of the relationships with environmental and hydrological factors (Jiang et al., 2013; Monk et al., 2012) and affect biodiversity patterns in regions with high species diversity (Heino et al., 2007). Some species exhibit greater heterogeneity in habitat preferences and response to disturbances in comparison to the family's average characteristics (e.g. water quality and flow preferences of species belonging to the Hydropsychidae family; Lenat and Resh, 2001). Thus, finer-level taxonomy (e.g.

identification at the genus level only in families with a wide intrafamilial variation in environmental tolerance; Chessman et al., 2007) may yield more accurate results in macroinvertebrate analyses.

Contrary to the last expectation, the CWM-RDA analysis reported a slightly lower total explained variation in diatom communities when compared to the taxonomy-based RDA. It could be related to the number of diatoms' traits (9 traits divided into 3 categories), which is much lower than the number of taxa (95 diatom species), resulting in considerably lower total inertia in the trait-based analysis relative to taxonomy (Heino et al., 2007). This difference was less pronounced in macroinvertebrate (11 trait groups, 60 taxa) and fish (6 trait groups, 38 taxa) assemblages, in which trait-based datasets revealed higher total explained variation than when using taxonomy.

2.4.4 Implications for investigating global change effects on rivers and mitigation actions

This study has shown that shifts in biological communities were significantly linked to temperature, precipitation, hydrological indices, agriculture, geology, and slope (i.e. a proxy for the position along the river network). These findings result promising to forecast and anticipate the potential effects of global change on river biota since these dynamic variables are greatly related to the main sources of change (i.e. climate, LULC, and hydrological changes) and will help to focus future research priorities. Specific study designs and ad-hoc experiments accounting for the effect of these dynamic variables are paramount to disentangling cause-effect relationships and cascade effects on ecosystem functioning and, ultimately, on the ecosystem services that rivers provide.

The three biotic groups, which represent the three main trophic levels on the riverine food webs, presented asymmetric responses to environmental variables. Diatoms and macroinvertebrates showed to be more sensitive to climate- and LULC-related factors, indicating that these communities may be good indicators of the ecological effects of land use (e.g. water quality deterioration, organic pollution, nutrients enrichment; Guse et al., 2015) and climatic changes (e.g. changes in temperature and precipitation trends; Timoner et al., 2020). Hydrology had an important contribution to fish community variability, suggesting that they may be good indicators of flow alteration and habitat connectivity (Geist and Hawkins, 2016). At the same time, variables related to geology and the position along the river network (i.e. upstream-downstream gradient) showed essential contributions to community shifts in the three biotic groups. The upslope gradient can also be

useful to delimitate climate-sensitive zones in which species or traits rapidly respond to climatic conditions and, therefore, efficiently monitor climate change impacts (Bässler et al., 2010; Shah et al., 2015).

Finally, the results obtained in this study are not only important to increase the understanding of the effects of global change on fluvial ecosystems, but also to inform integrated catchment management and to guide policy development aiming at mitigating these effects. For instance, many authors (e.g. Álvarez-Cabria et al., 2017; Ladrera et al., 2019; Lammert and Allan, 1999; Segurado et al., 2018; Silva et al., 2018) have already reported significant changes in riverine biological communities due to agriculture and its impacts (e.g. nutrient enrichment, sediment deposition, water abstraction, riparian habitat degradation). Thus, strategies such as conserving wetlands and riparian zones, which are capable of regulating the energy and material transfer between the terrestrial and aquatic ecosystems, can restore the river's natural capacity to buffer impacts caused by LULC, hydrological alteration, and climate change (Lind et al., 2019; Palmer et al., 2008; Pusey and Arthington, 2003). In this regard, Spain, a climate change hotspot (IPCC, 2021), dedicates around 17 million hectares to croplands (23% of them are irrigated; MAPAMA, 2021), making this activity a key economic engine of the country. Hence, to avoid the long-term decline in riverine biodiversity, adaptation strategies and water policy should account for the actual stressors affecting river ecosystems and avoid partial or highly local solutions. Nature-based solutions integrated into a landscape network should be strongly encouraged to help buffer changes in water temperature, hydrology, and diffuse pollution from agroforestry activities.

2.5 Supplementary Material

Table S2.1 Explanatory variables selected with a forward selection procedure (double-stopping criterion) using redundancy analysis (RDA) for taxonomy and community-weighted mean trait values (CWM-RDA) for traits. The coefficient of determination (R^2) of each variable and the accumulative adjusted coefficient of determination (R^2_{adj}) are shown. Analyses were performed with 9999 permutations and $\alpha = 0.05$. P-values are represented as $^{***} \leq 0.001$; $^{**} \leq 0.01$; $^{*} \leq 0.05$. Variables in green correspond to climate, in blue to hydrology, in red to LULC, in orange to geology, and grey to topography.

	Diatoms			Macroinvertebrates			Fish		
		R^2	R^2_{adj}		R^2	R^2_{adj}		R^2	R^2_{adj}
Taxonomy	TEMP	0.052	0.047***	PREC	0.043	0.041***	$SI_{NAT}2$	0.078	0.075***
	SLP	0.028	0.069***	TEMP	0.040	0.079***	CALC	0.067	0.138***
	SLIC	0.026	0.09***	ELEV	0.021	0.098***	ELEV	0.047	0.182***
	ELEV	0.016	0.102***	SLP	0.020	0.117***	CONG	0.033	0.213***
	$SI_{NAT}4$	0.013	0.11***	CALC	0.023	0.138***	$SI_{NAT}1$	0.025	0.235***
	$SI_{NAT}1$	0.012	0.117**	$SI_{NAT}1$	0.013	0.149***	AREA	0.028	0.261***
	$SI_{NAT}3$	0.013	0.126***	CONG	0.010	0.157***	SSH	0.016	0.275***
	CNF	0.011	0.132**	AGR	0.008	0.163***	$SI_{NAT}4$	0.014	0.286***
	PAS	0.009	0.137**	PAS	0.007	0.169***	SLP	0.014	0.298***
	PLT	0.009	0.141*	VOLC	0.006	0.173***	TEMP	0.013	0.308***
	OUT	0.009	0.145*	URB	0.005	0.176**	TEMP	0.012	0.318***
	SDIM	0.008	0.149*	DEN	0.005	0.179**	BLF	0.010	0.325***
				SLTE	0.005	0.182**	OUT	0.009	0.333***
				AREA	0.005	0.185**	CNF	0.008	0.338**
				CNF	0.004	0.188**	URB	0.007	0.343**
				SAND	0.004	0.19*	$SI_{NAT}3$	0.007	0.347**
							AGR	0.006	0.351*
							VOLC	0.005	0.354*
							PLT	0.005	0.356*
							EVT	0.005	0.359*

Continued on next page

Table S2.1 – *Continued from previous page*

	Diatoms			Macroinvertebrates			Fish		
		R^2	R^2_{adj}		R^2	R^2_{adj}		R^2	R^2_{adj}
Traits	AGR	0.058	0.052**	CALC	0.046	0.043***	TEMP	0.243	0.241***
	BLF	0.034	0.081*	AGR	0.048	0.090***	SSH	0.092	0.330***
	SDIM	0.023	0.1*	ELEV	0.024	0.111***	$SI_{NAT}1$	0.053	0.381***
	URB	0.034	0.129*	TEMP	0.022	0.131***	CNF	0.044	0.424***
				$SI_{NAT}1$	0.015	0.144***	CALC	0.020	0.442**
				PAS	0.013	0.156***	AGR	0.023	0.464**
				SLP	0.011	0.165***	CONG	0.028	0.490***
				VOLC	0.007	0.17**	TEMP	0.013	0.501*
				URB	0.007	0.175**	OUT	0.024	0.524***
				CNF	0.007	0.18**	PLT	0.008	0.531*
				AREA	0.006	0.185*	$SI_{NAT}3$	0.008	0.538*
				SLIC	0.006	0.189**	AREA	0.010	0.546*
				TEMP	0.004	0.192*			

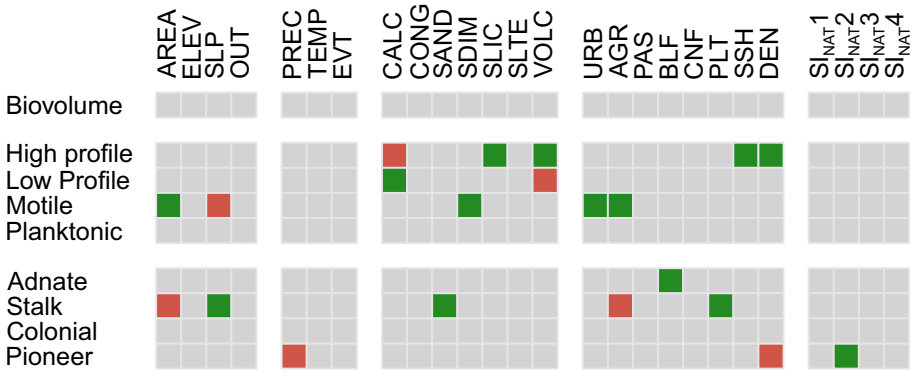


Figure S2.1 Fourth-corner correlations between diatom traits (rows) and environmental variables (columns; see Table 2.1 for codes). Grey boxes indicate non-significant correlations (p>0.05); red boxes indicate negative significant correlations; green boxes indicate significant positive correlations.

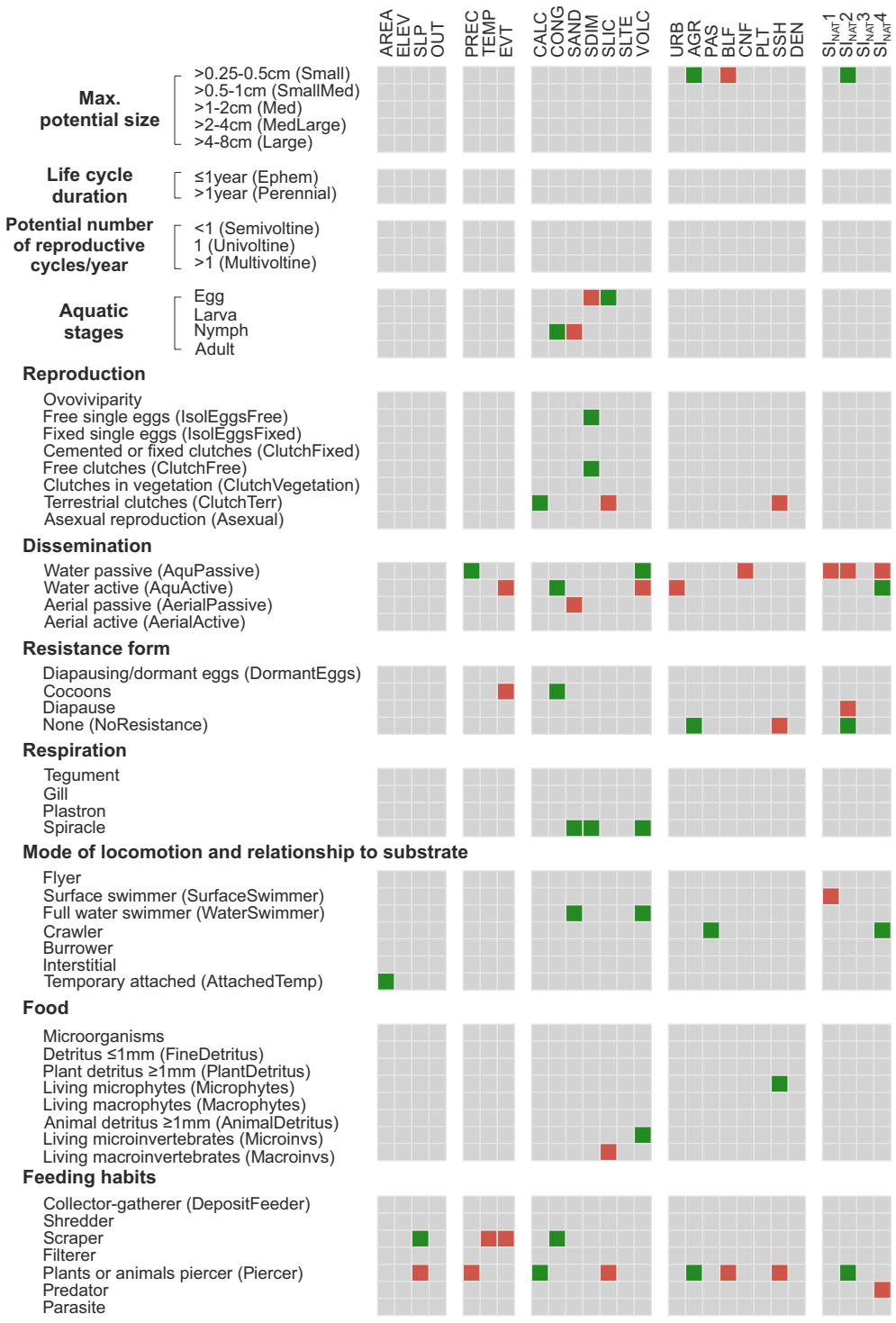


Figure S2.2 Fourth-corner correlations between macroinvertebrate traits (rows) and environmental variables (columns; see Table 2.1 for codes). Grey boxes indicate non-significant correlations (p>0.05); red boxes indicate negative significant correlations; green boxes indicate significant positive correlations.



Figure S2.3 Fourth-corner correlations between fish traits (rows) and environmental variables (columns; see Table 2.1 for codes). Grey boxes indicate non-significant correlations (p>0.05); red boxes indicate negative significant correlations; green boxes indicate significant positive correlations.

Chapter 3

Large-scale spatial patterns of riverine communities: Niche versus geographical distance

This chapter is an edited version of the research article published in the journal Biodiversity and Conservation by Cássia Rocha Pompeu, Francisco J. Peñas, and José Barquín in 2022 with the title "*Large-scale spatial patterns of riverine communities: niche versus geographical distance*". DOI: 10.1007/s10531-022-02514-6.

Abstract

Freshwater ecosystems are among the most threatened ecosystems on Earth. Effective conservation strategies are essential to reverse this trend and should be based on sound knowledge of biodiversity patterns and the main drivers structuring them. In this study, we investigated the role of environmental and dispersal-connectivity controls on freshwater diatom and fish communities' variability. We used 441 biological samples obtained from Spanish biomonitoring datasets, which cover a highly variable environmental gradient across the national river network. We compared the taxonomic and trait-based spatial dependency of the two biotic groups using distance-decay relationships and variation partitioning with spatially constrained randomisations. Our findings showed that most of the diatoms and fish biological variation were attributed to pure spatial and spatially structured

environmental variation. Compared to diatoms, fish community composition presented a stronger spatial dependency, likely because of their weaker dispersal ability. In addition, broad-scale environmental characteristics showed a higher predictive capacity for fish assemblages' variation. Trait-based similarities presented lower spatial dependency than taxonomic datasets, indicating that they are less susceptible to dispersal-connectivity effects. These findings contribute to understanding the mechanisms underlying river community assembly at large spatial scales (i.e. at and beyond the river network) and point out the importance of dispersal-connectivity processes, which are usually neglected in traditional niche-based biomonitoring programmes but can influence their outcomes (e.g. masking the detection of anthropogenic impacts). Therefore, the integration of the dispersal-connectivity component, as well as information on organisms' dispersal abilities, are crucial when establishing effective conservation objectives and designing biomonitoring strategies.

3.1 Introduction

Streams and rivers sustain a broad range of habitats and biodiversity, supporting the delivery of valuable ecosystem services for human societies (Grizzetti et al., 2016; Harrison and Dobson, 2008; Vörösmarty et al., 2010). Despite this, they face severe anthropogenic threats worldwide, such as effluent discharge, introduction of exotic species, and flow regime alteration with dams and reservoirs (Dudgeon et al., 2006; Reid et al., 2019). These increasing pressures do not change only the primary drivers defining riverine species' niche (e.g. temperature, water chemistry, benthic substrate, river morphology), but also lead to alterations in the connectivity of habitats, affecting their spatial dynamics at regional scales (McCluney et al., 2014).

To date, global trends in riverine ecosystem degradation call for urgent implementation of sustainable resource management, which should be underpinned by the most updated understanding of cause-effect relationships between anthropogenic impacts, biodiversity, and ecological processes (Dudgeon et al., 2006). In this regard, water management decisions are informed by cost-effective biological assessments (Bonada et al., 2006), which are required by the European Water Framework Directive (WFD; European Commission, 2000) and environmental legislation elsewhere, e.g. National Water Initiative in Australia (Australian Government, 2007) and the Clean Water Act in the USA (92nd U.S. Congress, 1972). However, few monitoring programmes and biotic indices have considered regional factors (e.g. connectivity and dispersal) that might affect the distribution of communities (but see Cid et al., 2020), often focusing only on niche-based processes and detecting anthropogenic impacts

based on the deviation from reference conditions (Downes et al., 2002; Siqueira et al., 2014).

Recent advances in spatial ecology look at the influence of the riverine network structure on biological patterns and processes shaping metacommunities (Altermatt and Fronhofer, 2018; Erős and Lowe, 2019; Tonkin et al., 2018), and new techniques (e.g. virtual watersheds; Barquín et al., 2015) facilitate the design and improvement of monitoring approaches (Heino et al., 2015; Siqueira et al., 2014). Riverine biological assemblages differ in their dispersal abilities and ecological preferences, and, at large spatial extents (i.e. scale at which most monitoring programmes are designed), they can be shaped by different factors. For example, good dispersers (e.g. diatoms, flying macroinvertebrates) are usually driven by environmental filtering (Astorga et al., 2012; Hájek et al., 2011), while weak dispersers (e.g. fish) are more constrained by geographical distances and connectivity among river reaches (Shurin et al., 2009). In this context, body size seems to be a good proxy of the dispersal capacity and, as such, an important biological characteristic to evaluate the contribution of environmental versus spatial controls on determining riverine community structure and composition (Astorga et al., 2012; De Bie et al., 2012). Moreover, functional traits have been recognised as an alternative to taxonomy, as they should be more dependent on environmental filters than on spatial constraints and could allow mechanistic interpretations of stressor's impacts (Chen and Olden, 2018; Gayraud et al., 2003; Menezes et al., 2010; Vandewalle et al., 2010).

The distance-decay relationships (DDR; Nekola and White, 1999) have been applied in a range of environments, organisms, and spatial extents (Brown and Swan, 2010; Soininen et al., 2007) to investigate geographical distance effects on communities, and provide important insights on spatial dynamics, especially for fragmented and highly dynamic freshwater habitats (Cañedo-Argüelles et al., 2015; Cid et al., 2020). The decay of community similarities over geographical distances can be first attributed to niche differences, as environmental variables tend to be spatially autocorrelated (Legendre and Fortin, 1989; Nekola and White, 1999) and spatial processes (e.g. organisms' dispersal, spatial configuration, isolation of habitats, mass effect; Hubbell, 2001; Soininen et al., 2007) also contributing to it. Likewise, variation partitioning is a quantitative method frequently used in ecology to assess the likelihood of predictors (e.g. environment, space) in explaining community patterns (Peres-Neto et al., 2006). It allows partitioning the variance into uniquely and jointly explained fractions and inferring underlying community assembly processes (Brown et al., 2017; Diniz-Filho et al., 2012). However, since dependency between biological communities, environment, and space is extremely common, the method should be

applied carefully as it can fail to control for spatial autocorrelation biases, generating spurious correlations (Gilbert and Bennett, 2010; Tuomisto et al., 2012).

To our knowledge, the importance of environmental and dispersal-connectivity factors in determining the taxonomic and functional structure of multiple river biological communities at large spatial scales (i.e. multiple neighbouring river networks) has been rarely evaluated simultaneously (but see Henriques-Silva et al., 2019; Keck et al., 2018). Thus, in this study, we selected diatoms and fish, organisms with contrasting dispersal abilities, to investigate the relative importance of environmental- (i.e. niche) and spatial-related (i.e. dispersal-connectivity) mechanisms on community composition based on taxonomy (C-TX) and community structure based on functional traits (C-FT) across continental Spain. We expect that (1) the importance of environmental and dispersal-connectivity processes will differ between diatoms and fish, with (2) dispersal-connectivity processes being a key determinant for fish C-TX while (3) environmental factors being more relevant for diatom C-TX (i.e. dispersal ability and spatial control inferred from body size; De Bie et al., 2012). In addition, we expect that (4) C-FT will have a lower spatial dependency in comparison to C-TX, as functional traits should be more responsive to niche characteristics than to dispersal-connectivity dynamics (Hoeinghaus et al., 2007). Finally, we will highlight the implications of our findings for riverine biomonitoring programmes.

3.2 Methodology

3.2.1 Study area

The study area comprises continental Spain (Figure 3.1), a country with great environmental variability within the Atlantic and the Mediterranean zones. The relief is characterised by an extensive high inland plateau (average of 650 m.a.s.l.) surrounded by relatively high mountain ranges: the Cantabrian Cordillera in the north, the Iberian System in the east, and the Sierra Morena in the south. Outside this central plateau, the Pyrenees in the northeastern border and the Betico system in the southeast are the highest mountain ranges, with peaks reaching 3,400 m.a.s.l. (Peñas and Barquín, 2019; Rivas-Martínez et al., 2004).

This complex orography plays a crucial role in defining the climate and hydrography. Five Köppen-Geiger climate zones are distributed along the area: mountain climate in high altitudes, with cold winters and abundant precipitations (annual averages reaching 3,000 mm); the oceanic climate in the northwest region;

hot steppe climate in the southeast, with minimum rainfall in Spain (annual averages lower than 150 mm); and several Mediterranean climate variations, with dry and hot summers (AEMET and IMP, 2011; Rivas-Martínez et al., 2004). Thus, the twelve main catchments in the study area are conditioned by the combination of relief and climatic features, causing high variability in hydrological patterns (Peñas et al., 2014).

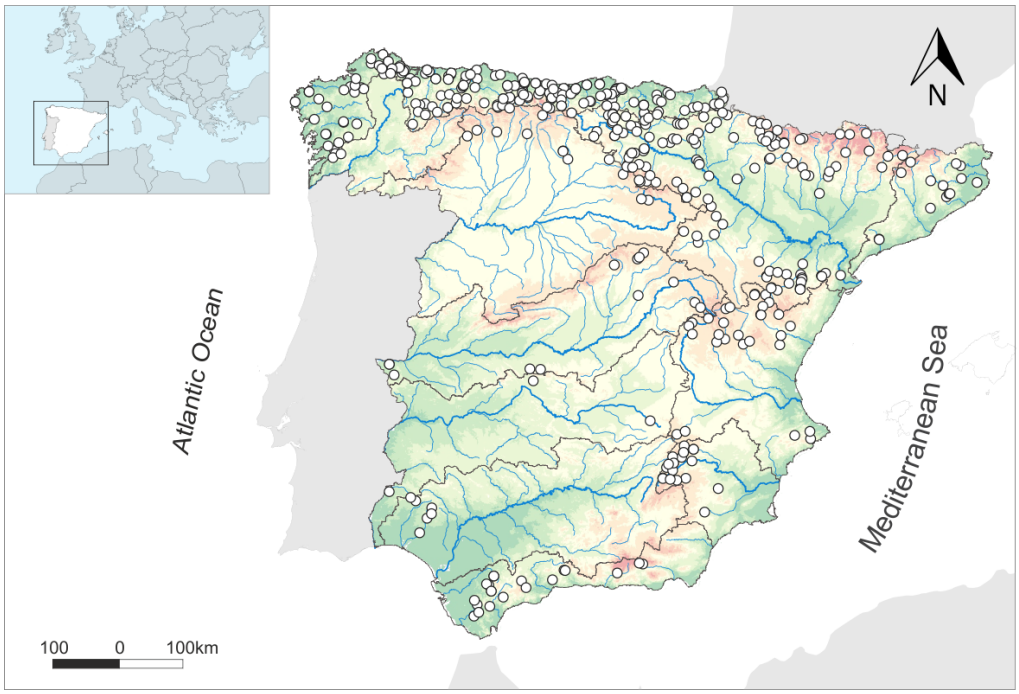


Figure 3.1 Study area (505,990 km^2) and sampling site selection, comprising a total of 441 samples.

3.2.2 Characterisation of biological communities

In this study, we used the biological monitoring database NABIA (Spanish Royal Decree 817/2015 on Water Policy; BOE, 2005) compiled and provided by the Spanish Ministry for the Ecological Transition (MITECO). This database provides information on biological indicators from monitoring campaigns carried out since 2008 for assessing the ecological status and water quality of all water bodies in the country in compliance with the WFD (European Commission, 2000). Field surveys followed standard sampling protocols to compute biotic indices, such as the Specific Pollution Sensitivity Index for diatoms (IPS; CEMAGREF, 1982) and the Cantabrian Fish Index (CFI; De Bikuña et al., 2017).

From the NABIA database, we selected diatom and fish samples collected between May and October to maximise the spatial coverage while reducing "noise" caused by intra-annual variability, as done in previous studies (e.g. Leathwick et al., 2005). The

communities were surveyed annually between summer and early autumn depending on meteorological and hydrological conditions. Regarding diatoms, following the standard procedure UNE-EN13946:2014 (CEN, 2014a), five to ten cobbles were selected randomly from the benthos (ca. 100 cm^2 of exposed surface area) at a depth of ca. 10 cm to ensure that they were not exposed to air in the previous four weeks and that they were placed in the euphotic zone. Areas of heavy shade and close to the bank were avoided, as well as zones of slow current (approx. $\leq$ 20 $cm.s^{-1}$). Then, the upper part of the substratum was scrubbed with a dishwasher brush. Aliquots of the diatom samples collected were digested with hydrogen peroxide and permanent slides were mounted with Naphrax®. The material was decanted in a sample bottle and preserved using formaldehyde. From each sample, 400 diatom valves were identified in laboratory using a microscope (1000× magnification) at the lowest feasible taxonomic level, according to the standard procedure UNE-EN 14407:2014 (CEN, 2014b).

For fish communities, a single-pass sampling was performed using a portable electric fishing device (current generation 300-600 V, up to 1.5 A) in a representative area of the wadeable stream (10 times the average width of the stream and a minimum of 100 m^2), following the standard procedure UNE-EN 14011:2003 (CEN, 2003). After the pass removal, the captured fish were kept in oxygenated boxes, anaesthetised, counted, and identified at the species level. The fish were then released back to the stream alive after a recovery period.

Diatoms and fish were identified at the species level (see Tables S3.3 and S3.4 in Supplementary Material). Although macroinvertebrates are also widely used in the Spanish bioassessment programmes, we did not include the available samples due to their coarse taxonomic resolution (i.e. mainly at the family level). The family-level identification of macroinvertebrates is required for computing the Iberian Biological Monitoring Working Party (IBMWP) index (Alba-Tercedor et al., 2002).

Only samples surveyed in river reaches catalogued as "good" or higher ecological status were retained. The ecological status was determined using biological, physico-chemical, and hydromorphological characteristics and reference conditions for surface waters, as specified in the Spanish Hydrological Plan 2015-2021 within the European WFD (BOE, 2005). Further, only sites unaffected by local anthropogenic pressures such as dams, embankments, or significant water abstractions upstream were kept for subsequent analyses. This site selection covering only minimally disturbed streams reduced the confounding effect of other stressors, such as water pollution, hydromorphological pressures, or the presence of dams and reservoirs. After applying these criteria, we retained 177 sampling sites for diatoms and 264 for fish (Figure 3.1).

The abundance of taxa was averaged in sites surveyed for multiple years to obtain a site-specific assemblage composition, following previous studies (Filker et al., 2016; Paavola et al., 2003). Due to the high number of diatom species identified, we eliminated rare taxa (those representing less than 2% of total sampled individuals when taking into account the total number of occurrences; Lavoie et al., 2009). Then, in the two biotic groups, C-TX was determined based on the presence or absence of each taxon.

To characterise C-FT, we assigned diatoms' species to ecological guilds, size (biovolume), and life forms according to Rimet and Bouchez (2012) (see Table B.1 in Appendix B). For fish, we assigned the species to trait categories describing body size, feeding habits, tolerance to stressors, habitat use, and migration (see Table B.3 Appendix B), based on Iberian species information obtained from Doadrio (2002), Schmidt-Kloiber and Hering (2015), and Cano-Barbacil et al. (2020). It is important to note that trait categories can overlap and species may belong to multiple trait categories (e.g. *Amphora pediculus* is considered, at the same time, a low profile, pioneer, adnate, and non-colonial species; *Phoxinus bigerri* is a non-migratory, omnivorous, rheophilic fish species inhabiting the water column). Then, for both organisms, the relative abundance corresponding to each trait category was calculated based on the abundance of the taxa contributing to it.

3.2.3 River network and environmental information

River reaches were characterised using hydrological and environmental information integrated within the virtual watersheds composing the Spanish river network (Barquín et al., 2015) constructed using flow direction inferred from a 10-meter digital elevation model. Environmental variables describing topography, climate, land use and land cover, and geology (Table 3.1) were obtained from national and regional databases (Peñas and Barquín, 2019).

To characterise the flow regime, we used a set of non-correlated synthetic hydrological indices (SI_{NAT}). They were calculated based on the normalised daily flow series recorded in natural flow gauges (CEDEX, 2022) and predicted to all river reaches in the country using Random Forest models (Peñas and Barquín, 2019). The four SI_{NAT} (Table 3.1) explained 84% of the total hydrological variance and set the critical natural hydrological patterns in continental Spain. They can be individually interpreted according to the HI with the highest contribution (Table A.2 in Appendix A; further details in Peñas and Barquín, 2019).

Table 3.1 Catchment-scale environmental variables describing topography, climate, land use and land cover, geology, and hydrology in the diatoms (n=177) and fish (n=264) sampling sites. Variables refer to the mean catchment values upstream of the sampling site.

Description	Diatoms	Fish
	Mean ± sd (range)	Mean ± sd (range)
Catchment size (km^2)	226 ± 502.2 (0.9 - 4845)	225.6 ± 410.1 (1.6 - 4845)
Distance to river outlet (km)	394 ± 272.1 (0 - 915)	176.3 ± 252.2 (0 - 928.3)
Elevation above sea level (m)	653.1 ± 351.7 (1 - 1734)	372.8 ± 339 (1 - 1.435.3)
Slope in segment (0 - 1)	0.3 ± 0.1 (0 - 0.7)	0.4 ± 0.2 (0.1 - 1.0)
Annual precipitation (mm)	864.3 ± 327.4 (329.3 - 1796.3)	1132.1 ± 342.6 (455.9 - 1947.1)
Annual temperature (°C)	11.9 ± 2.6 (6.2 - 18)	11.8 ± 2.3 (5 - 18.6)
Annual evapotranspiration (mm)	477.2 ± 66.1 (294.3 - 684.2)	511.4 ± 75.5 (318.3 - 726.8)
Fraction occupied by urbanisation	0.0 ± 0.0 (0 - 0.1)	0.0 ± 0.0 (0 - 0.1)
Agriculture	0.1 ± 0.2 (0 - 0.6)	0.1 ± 0.1 (0 - 0.6)
Pastures	0.2 ± 0.2 (0 - 0.8)	0.2 ± 0.1 (0 - 0.7)
Broadleaf forests	0.2 ± 0.2 (0 - 0.9)	0.2 ± 0.2 (0 - 0.8)
Coniferous forests	0.1 ± 0.2 (0 - 1)	0.1 ± 0.2 (0 - 0.8)
Heathlands, shrubs	0.2 ± 0.2 (0 - 0.7)	0.3 ± 0.2 (0 - 0.7)
Denuded areas	0 ± 0.1 (0 - 0.7)	0.1 ± 0.1 0 - 0.7)
Fraction occupied by calcareous rocks	0.5 ± 0.4 (0 - 1.0)	0.4 ± 0.4 (0 - 1.0)
Conglomerate rocks	0.3 ± 0.3 (0 - 1.0)	0.2 ± 0.3 (0 - 1.0)
Sedimentary rocks	0 ± 0.1 (0 - 0.7)	0 ± 0.0 (0 - 0.4)
Siliceous rocks	0.1 ± 0.3 (0 - 1.0)	0.2 ± 0.3 (0 - 1.0)
Volcanic rocks	0 ± 0.1 (0 - 0.8)	0.1 ± 0.3 (0 - 1.0)
$SI_{NAT}1$	-1.49 ± 0.62 (-1.49 - 1.51)	0.10 ± 0.45 (-1.55 - 1.59)
$SI_{NAT}2$	0.21 ± 0.71 (-1.21 - 1.42)	-0.30 ± 0.67 (-1.40 - 1.40)
$SI_{NAT}3$	-0.04 ± 0.49 (-1.79 - 1.10)	-0.14 ± 0.48 (-1.42 - 0.95)
$SI_{NAT}4$	0.24 ± 0.39 (-0.81 - 1.44)	-0.07 ± 0.42 (-1.09 - 1.21)

3.2.4 Data Analyses

We used DDR (Nekola and White, 1999; Soininen et al., 2007) to assess the effects of geographical distances and environmental characteristics on diatoms and fish assemblages. We analysed the dispersal-connectivity and environmental control in both biotic groups and their C-TX and C-FT using the DDR linear regression

coefficients (slope), which described the rate of species turnover, and the initial similarities (intercept), which described the species turnover at small spatial extents (Astorga et al., 2012).

The DDR was estimated regressing biological similarities as a function of geographical and environmental distances, with the Jaccard index for C-TX pairwise biological communities similarities and Bray-Curtis distances for C-FT. Since our study area was composed of multiple unconnected river networks, we computed Euclidean distances based on samples' latitude and longitude, ranging from 1 to 1,031 km. Similarly, to compute environmental distances, the environmental variables (Table 3.1) were first standardised (zero mean and unit variance), as they had different measurement units and ranges, and the pairwise Euclidean distances between all sites were calculated. We used partial correlation analysis (Legendre and Legendre, 2012) to factor out the effect of the geographical distances on environmental variables and test for independent correlations between environmental distances and biological similarities. Given the different ranges described by geographical and environmental distances, we re-scaled both distances to values between 0 and 1.

Then, to quantify and compare the relative contribution of environmental and geographical distance-related processes to the diatom and fish C-TX and C-FT variability, we used variation partitioning (Peres-Neto et al., 2006). The biological variation was decomposed into four fractions: (1) the variation explained uniquely by non-spatial environmental variation, i.e. species variation explained by the environmental dataset (Table 3.1: topography, climate, geology, LULC, and hydrology) independently of any spatial structure, (2) uniquely by spatial patterns (i.e. geographical distance among sites) that are not shared by the environmental dataset, (3) by the spatial patterns in community data that are shared by the environmental dataset (i.e. shared or spatially structured environmental variation), and (4) the unexplained variation (Borcard et al., 1992). We used Moran's eigenvector maps (MEMs; Dray et al., 2006) as a proxy for the biological communities' spatial distribution patterns (i.e. geographical distances). The MEMs are linearly independent vectors capable of describing a wide range of spatial scales (Griffith and Peres-Neto, 2006) and are linked to a spatial weighting matrix (SWM), which determines the spatial relationships between the sampled sites (Bauman et al., 2018a). We tested three graph-based schemes (Gabriel graph, relative neighbourhood graph, and minimum spanning tree) with two weighting matrices (binary and linearly decreasing as a function of pairwise-site distances) as potential SWM (Benone et al., 2020). All positively spatially correlated MEMs for all SWM candidates were computed, as a subset of MEMs is not able to remove the spatial autocorrelation from model residuals and may lead to inflated type I error of the

pure spatial fraction (Clappe et al., 2018; Peres-Neto and Legendre, 2010). Then, an optimisation procedure (Bauman et al., 2018b) selected the SWM which MEMs yield to the maximisation of the adjusted R^2 (see detailed explanation in Section 3.5.1 in Supplementary Material). Regarding environmental predictors, we ran a forward selection procedure to retain the relevant environmental variables with a double-stopping criterion (Blanchet et al., 2008) to avoid overestimation of explained variance.

Finally, Moran Spectral Randomisation (MSR; Clappe et al., 2018; Wagner and Dray, 2015) was applied to correctly account for spatial autocorrelation in the variation partitioning. This method performs spatially constrained randomisations in variation partitioning and maintains the data's spatial characteristics, avoiding type I error inflation and removing spurious correlations (Clappe et al., 2018).

All statistical analyses were performed in *R* environment (R Core Team, 2020) using the packages `vegan` (Oksanen et al., 2019) and `adespatial` (Dray et al., 2020).

3.3 Results

3.3.1 Diatoms and fish assemblages

A total of 95 diatoms species were retained in our analyses (Table S3.3 in Supplementary Material). The most abundant species were *Achnanthidium minutissimum* (corresponding to 19.2% of all sampled diatoms), *Achnanthidium pyrenaicum* (14.2%), *Cocconeis euglypta* (8.1%), *Amphora pediculus* (4.5%), and *Achnanthidium lineare* (4.2%). Other genera, such as *Gomphonema* (corresponding to 9.4% of all sampled diatoms) and *Nitzschia* (5.4%), were also present in samples. Regarding their traits, the high-profile guild, stalked, and small-sized diatoms dominated most of the sampling sites.

Fish assemblages had a total of 38 species (Table S3.4 in Supplementary Material). The most abundant taxa were *Salmo trutta* (corresponding to 29.8% of all sampled fish), *Phoxinus bigerri* (28.7%), *Parachondrostoma miegii* (6.7%), *Salmo salar* (5.5%), and *Anguilla anguilla* (4.2%). Regarding their traits, migratory, sensitive to stressors, and large-bodied species dominated most of the sampling sites.

3.3.2 Similarity decay over geographical and environmental distances

The biological similarities decreased with geographical and environmental distances in both fish and diatom communities (Figure 3.2 and Table S3.1 in Supplementary Material). Diatoms presented lower initial similarities compared to fish, indicating higher beta diversity at small spatial scales. Fish biological similarities showed steeper decay rates when compared to diatoms (1.5 and 2.5 times steeper over geographical distances for C-TX and C-FT, respectively; Figure 3.2a, d), indicating a higher explanatory capacity of the environmental variables and spatial patterns for fish. Fish C-TX similarities presented a large number of "zero" (24% of samples) or "one" (5% of samples) values, showing that these assemblages had no species in common, or shared all species, respectively. In both organisms, the C-FT (Figure 3.2, in grey) showed lower decay rates (-57% in diatoms and -38% in fish), higher initial similarities and higher scattering over geographical distances when compared to the C-TX, indicating that geographical distances exerted a lower control on community similarity when using functional traits.

The geographical and environmental distances among samples showed positive correlations (see Figure S3.1 in Supplementary Material), suggesting that environmental characteristics were spatially correlated. In both organisms, the DDR over environmental distances (Figure 3.2b, e), when compared to the DDR over geographical distances, showed higher initial similarities and decay rates (an increase of 100% for diatoms and 60% for fish, on average). In contrast, after splitting out the spatial component (Figure 3.2c, f), the decay rates were 54% higher for diatoms and 7% lower for fish in relation to the DDR over geographical distances, indicating that spatial patterns exerted an important control on fish assemblage variation.

3.3.3 Contribution of environmental versus spatial variables

The variation partitioning (Figure 3.3 and Table S3.2 in Supplementary Material) showed that environmental variables and MEMs explained larger fractions of the biological variation in fish assemblages (50.4%, on average) when compared to diatoms (31.1%), in agreement with the DDR analysis. The shared fraction between environment and spatial patterns (i.e. spatially structured environmental fraction) corresponded to the major proportion of explained variation in most cases (see also Section 3.5.1 in Supplementary Material).

Environmental variables and MEMs, together, accounted for 25.5% and 36.7% of the diatoms C-TX and C-FT variability, respectively (Figure 3.3a, b). The

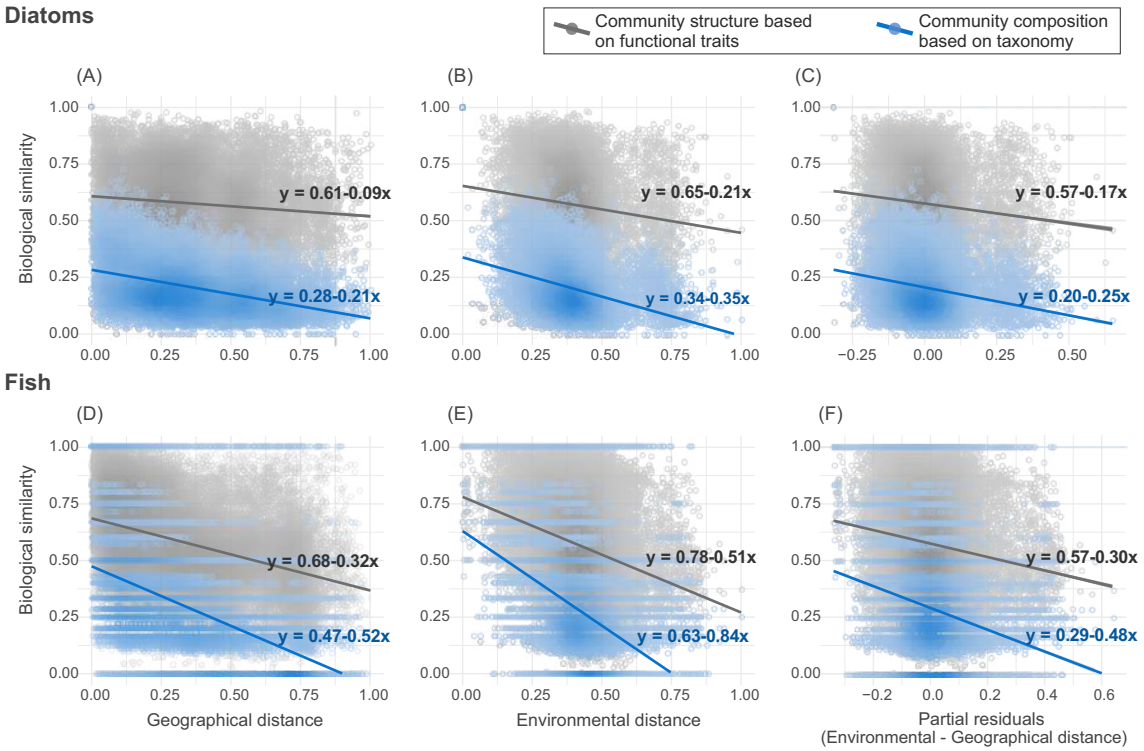


Figure 3.2 Biological similarity decay for diatoms (A-C) and fish (D-F) over geographical distances, environmental distances, and environmental partial residuals after removing the spatial component. We used Jaccard index among pairs of samples for taxonomical community composition (in blue) and Bray-Curtis similarities for trait-based community structure (in grey). Equations were obtained with simple linear regression (see Table S3.1 in Supplementary Material).

contribution of the pure spatial component was similar in both cases, corresponding to 52.4% of the total explained variation in diatom C-TX and 52.8% in C-FT. The spatially structured environmental contribution was slightly higher when C-FT was considered (47.2% of the total explained variation in comparison to 45.7% for C-TX). The fraction of community variability attributed to the non-spatialised environment was negligible in both cases.

Environmental variables and MEMs, together, accounted for 54.3% and 46.6% of the explained variation in fish C-TX and C-FT variability, respectively (Figure 3.3c, d). In contrast to diatoms, the pure environmental fraction had small but significant contributions to fish C-TX and C-FT variability. When considering fish C-TX, the contribution of pure spatial and spatially structured environmental fractions were similar. In fish C-FT, the spatially structured environmental fraction explained twice the variation of the pure spatial fraction, suggesting that fish functional traits are less subject to dispersal-connectivity processes and are more responsive to environmental characteristics.

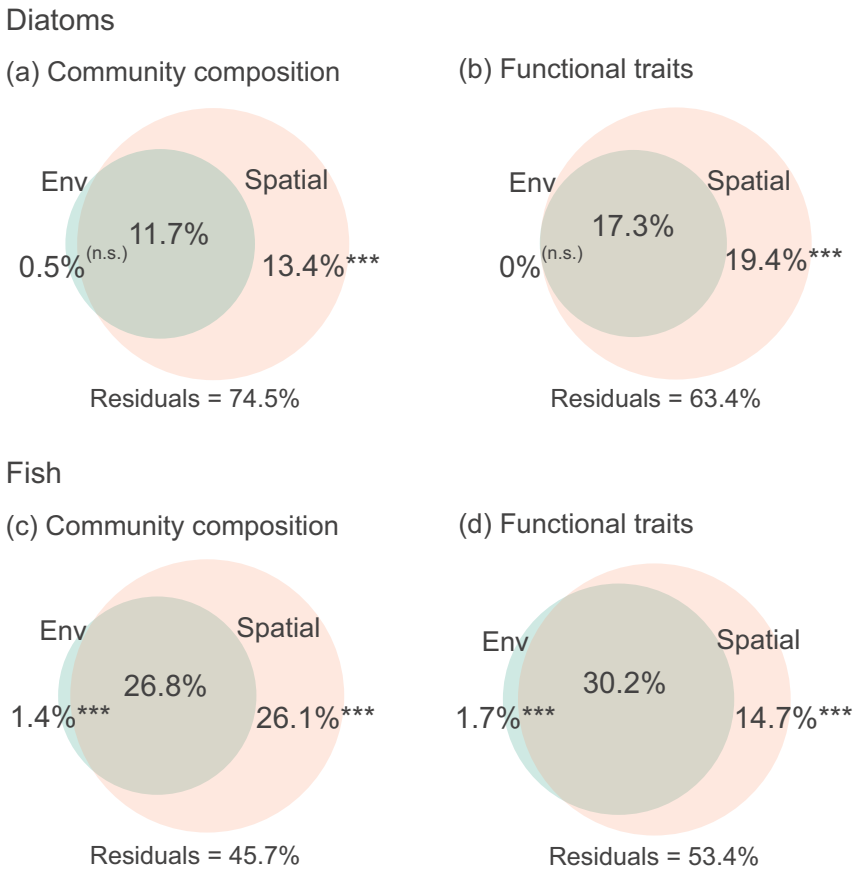


Figure 3.3 Variation partitioning of the environmental and spatial components in the taxonomic and trait-based communities of diatoms (A, B) and fish (C, D). Fractions represent pure environmental (Env), spatialised environmental (shared fraction), and pure spatial fraction (Spatial) after MSR corrections. The significance of testable fractions was determined with ANOVA of RDA models; p-values are represented as: *** ≤ 0.001; ** ≤ 0.01; * ≤ 0.05; n.s. not significant (see also Table S3.2 in Supplementary Material).

3.4 Discussion

This study indicates that environmental and spatial controls differ in their contribution to structuring riverine biological communities. Geographical distances exerted a higher control on fish (low dispersal abilities) than on diatom (high dispersal abilities) communities, while the non-spatially structured environment showed minimal contributions. Finally, community similarity based on functional traits showed a higher influence of environmental factors than geographical distances.

3.4.1 Differences between biotic groups: the effects of dispersal capacity on biological assemblages

Our findings showed a decrease in biological similarities over geographical and environmental distances, suggesting that diatom and fish assemblages are jointly

driven by niche (i.e. environmental filtering; Leibold et al., 2004) and spatial (i.e. distances and organisms' dispersal; Hubbell, 2001) processes. The differences observed in initial similarities, decay rates, and the fraction of variation attributed to environment and space indicated that environmental and distance-related dynamics share the controlling the biological variation in the two communities, in agreement with our first hypothesis and previous studies (Astorga et al., 2012; Brown and Swan, 2010; Keck et al., 2018; Leibold and Chase, 2018).

Geographical distances in the DDRs and the MEMs in the variation partitioning showed a higher control on fish assemblages in comparison to the non-spatialised environment, supporting our second hypothesis. The obligated aquatic dispersion through the river network and larger body size have been suggested to limit fish propagation and settlement along a larger range of habitat conditions (Cohen et al., 2003). These characteristics could contribute to the higher spatial control on fish C-TX found in this study and elsewhere (Astorga et al., 2012; Shurin et al., 2009). Besides, suitable environmental conditions in distant river reaches would not assure the colonisation by specific fish species, as connectivity (e.g. unconnected river basins, dams forming dispersal barriers) or the past biogeographical history have a key role in determining fish community patterns at large spatial scales (Leibold et al., 2010; Mazaris et al., 2010). These factors seem to be plausible explanations for the lower beta diversity and higher distance decay of fish communities.

Diatom C-TX showed a lower spatial control in comparison to fish, as demonstrated in the DDRs and variation partitioning analyses. These results agree with previous research (Finlay, 2002; Vilmi et al., 2016) and could be linked to the ecological characteristics of diatoms (i.e. smaller size, shorter generation times, larger populations, and aerial dispersion), which allow them to overcome geographical barriers and colonise sites with favourable conditions. However, since environmental variables and MEMs together explained relatively low amounts of diatom community similarities and the contribution of the pure environmental fraction was negligible, our findings could not support our third hypothesis. The low explanatory capacity of environmental variables for diatom C-TX, which has also been reported elsewhere (Keck et al., 2018; Liu et al., 2013), could be attributed to the large-scale definition of our environmental variables. Relevant local variables (e.g. water quality, physico-chemical characteristics, hydraulic and riparian conditions) that could be critical for determining diatom community composition (Hájek et al., 2011; Soininen et al., 2016, 2009) were not covered in our dataset. Furthermore, the complexity of diatom communities (i.e. a higher number of rare species in comparison to fish), biotic interactions, and random and stochastic processes (e.g. ecological

drift), which cannot be captured in our analyses, may also influence the community assembly dynamics (Hubbell, 2001; Vellend et al., 2014; Vilmi et al., 2017).

Community similarities based on functional trait dominance were less spatially dependent than community similarities based on taxonomic data. C-FT showed lower decay rates and higher scattering in both fish and diatom DDRs, and fish C-FT pure spatial contribution had a decrease of 16.5% in the total variation explained in relation to C-TX. These findings support our last hypothesis and suggest that trait-based approaches are more stable across large spatial extents and multiple biogeographical units, grouping the complexity of species composition into a reduced number of traits. Therefore, functional trait community structure should be less subject to geographical distances since trait filters act in a similar way across large ecoregions (Statzner and Bêche, 2010) and could provide more consistent ecological responses to environmental variation regardless of spatial distribution (Hoeinghaus et al., 2007). Our diatoms C-FT showed a higher contribution of pure spatial fraction when compared to C-TX; in contrast, their spatial control was similar in relation to the total explained variation. Previous studies (Jamoneau et al., 2018; Liu et al., 2013; Passy, 2007) also reported important spatial control on diatom C-FT, e.g. strongly attached growth forms are predominant in headwaters, while weakly attached forms are predominant in higher-order streams. However, rather than indicating the effect of spatial structuring in the species distribution, high pure spatial fractions can also be inflated by missing relevant spatially structured environmental variables (Diniz-Filho et al., 2012).

3.4.2 Implications for biomonitoring programmes

Our study supports the idea that geographical distance and environmental spatial autocorrelation (closer river reaches are more environmentally similar), which are rarely taken into account in riverine biomonitoring programmes (Cid et al., 2020; Siqueira et al., 2012), contributed significantly to explaining the variation in diatom and fish assemblages (i.e. organisms commonly used in biomonitoring). Our analyses incorporate river networks from 12 distinct river basins in Spain, suggesting that riverine communities have not only an important spatial dependency on the river network structure itself (Altermatt, 2013; Tonkin et al., 2018) but also across the multiple neighbouring river networks. Our results support the view that regional species pool and dispersal seem to be fundamental factors determining taxonomic differences in river biological assemblages, as has been shown elsewhere (Leibold and Chase, 2018; Viana and Chase, 2019).

These findings have important consequences for river biomonitoring and conservation. For example, the use of organisms with higher dispersal limitation, such as fish, in biomonitoring might be relevant to capture connectivity issues caused by human activities (e.g. dam construction), but might not be as promising for detecting changes in niche characteristics (e.g. hydro-morphological changes), as not all potential species may be present. Therefore, accurate detection of anthropogenic impacts can be compromised (Siqueira et al., 2014).

Another important implication of our results is that biomonitoring programmes may fail to detect restoration benefits if desired taxa are unable to reach new suitable locations; thus, the presence of protected or unaltered river reaches with source populations in the immediate vicinity of the restored sites (e.g. within a 5-km radius for potential recolonisation of macroinvertebrates; Sundermann et al., 2011) is crucial (Brown and Swan, 2010; Siqueira et al., 2014). This later issue reflects the need to incorporate multiple and well-connected river reaches in relatively good ecological status across the landscape matrix so that investment in recovery is actually successful. Finally, the spatial configuration of river typologies might also affect the establishment of reference conditions (e.g. 37 different river typologies and reference conditions identified over the Spanish river network; BOE, 2005). Reference conditions should cover relatively small geographical areas in order to reduce the effects of dispersal-connectivity processes on taxonomic differences, ensuring that the targeted organisms are able to reach and persist at all sites and allowing proper detection of species sorting dynamics (Heino et al., 2017).

Thus, dispersal-connectivity processes can have important effects on the performance of bioindicators and biomonitoring efforts, as different organisms' dispersal abilities and mass effects (intense dispersion) can mask anthropogenic disturbances or environmental factors controlling species distribution (Cid et al., 2020; Siqueira et al., 2012; Smucker and Vis, 2011). In order to reinforce river biomonitoring programmes and the design of effective conservation strategies, we encourage that potential spatial-related factors, such as organism's dispersal and habitat connectivity acting at and beyond the river network, should be considered in the selection of reference sites, targeted organisms, grain extent, and spatial scales (Bonada et al., 2006; Cid et al., 2020; Seymour et al., 2016).

The functional traits, when compared to taxonomic approaches, showed a lower spatial dependency in our study. This result suggests that functional traits would be more stable across biogeographical regions or multiple river networks, as they can provide valuable insights into the processes and mechanisms structuring biological assemblages (Usseglio-Polatera et al., 2000; Vandewalle et al., 2010), while species composition tends to respond to historical and spatial factors (Soininen et al., 2016).

Functional traits could also facilitate the development of river typologies and reference conditions often used in biomonitoring, as they are responsive to niche characteristics (Culp et al., 2011; McGill et al., 2006). Moreover, functional approaches would allow a better understanding of biodiversity, which is usually limited to taxonomic richness (Bêche and Statzner, 2009), and the development of holistic assessments of anthropogenic impacts (e.g. taking into account biotic interactions in a multi-trophic perspective; Aubin et al., 2013).

In summary, our findings indicate that environmental and dispersal-connectivity processes contributed to determining the structure and composition of the riverine biota in continental Spain. Both environmental and spatial aspects should be considered in national multi-scale biomonitoring programmes to assess riverine ecosystem health accurately. Functional approaches, besides their known advantages over taxonomy-based methods, can also complement biomonitoring efforts with consistent responses across broad spatial scales (i.e. when incorporating multiple ecoregions and biogeographic areas).

3.5 Supplementary Material

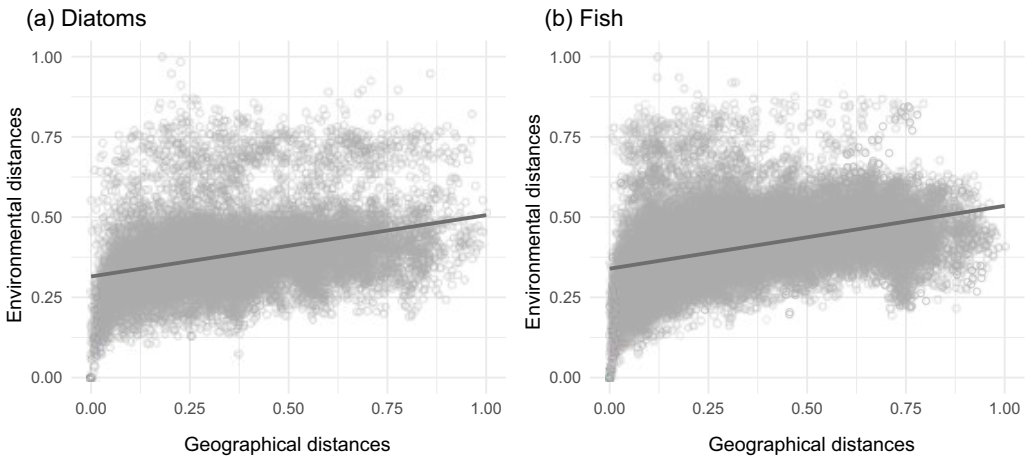


Figure S3.1 Geographical and environmental distances among samples (re-scaled to 0-1 values) showed a moderate positive correlation: 0.34 for (a) diatoms and 0.40 for (b) fish.

Table S3.1 Distance-decay relationship (DDR; Figure 3.2) parameters: coefficient of determination (R^2_{adj}), intercept, slope, and p-values.

	Similarity	Distance	R^2_{adj}	Intercept	Slope	p-value
Diatoms	Taxonomy	Geographical distance	0.137	0.282	-0.214	0.001
		Environmental distance	0.114	0.338	-0.347	0.001
		Partial residuals	0.051	0.205	-0.246	0.001
	Traits	Geographical distance	0.012	0.606	-0.088	0.001
		Environmental distance	0.022	0.654	-0.207	0.001
		Partial residuals	0.014	0.574	-0.174	0.001
Fish	Taxonomy	Geographical distance	0.136	0.282	-0.213	0.001
		Environmental distance	0.114	0.338	-0.344	0.001
		Partial residuals	0.051	0.205	-0.245	0.001
	Traits	Geographical distance	0.014	0.606	-0.093	0.001
		Environmental distance	0.021	0.650	-0.202	0.001
		Partial residuals	0.012	0.573	-0.165	0.001

Table S3.2 Statistical parameters of the ANOVA tests performed on the variation partitioning testable fractions (Figure 3.3).

	Similarity	Fraction	Degrees of freedom	F-value	*p*-value
Diatoms	Taxonomy	Pure Environmental	13	1.114	0.085
		Pure Spatial	85	1.275	0.001
	Traits	Pure Environmental	9	0.945	0.551
		Pure Spatial	84	1.489	0.001
Fish	Taxonomy	Pure Environmental	19	1.511	0.001
		Pure Spatial	126	1.954	0.001
	Traits	Pure Environmental	14	1.718	0.001
		Pure Spatial	125	1.455	0.001

Table S3.3 Mean abundance, standard deviation, and the number of occurrences (n) of diatom taxa across the selected sampling sites (n=177).

Code	Diatom taxa	Mean ± sd	n
ADMI	*Achnanthidium minutissimum*	75.99 ± 70.09	172
ADPY	*Achnanthidium pyrenaicum*	56.32 ± 80.47	124
CEUG	*Cocconeis euglypta*	32.25 ± 50.92	125
APED	*Amphora pediculus*	17.85 ± 34.69	109
ACLI	*Achnanthidium lineare*	16.57 ± 43.75	67
GPUM	*Gomphonema pumilum*	10.77 ± 23.26	92
COPL	*Cocconeis pseudolineata*	9.99 ± 34.46	48
NCTE	*Navicula cryptotenella*	8.46 ± 13.78	117
ECPM	*Encyonopsis minuta*	5.87 ± 15.2	53
GMIN	*Gomphonema minutum*	5.78 ± 19.54	67
CAEX	*Cymbella excisa*	5.74 ± 11.46	85
RSIN	*Reimeria sinuata*	5.59 ± 22.81	74
RABB	*Rhoicosphenia abbreviata*	5.49 ± 20.16	71
NFON	*Nitzschia fonticola*	4.92 ± 20.2	63
NTPT	*Navicula tripunctata*	4.91 ± 16.87	74
CPLI	*Cocconeis placentula var. lineata*	4.75 ± 18.63	51
ADAM	*Achnanthidium atomoides*	4.67 ± 27.27	29
FSAP	*Fistulifera saprophila*	4.48 ± 15.82	47
NIFR	*Nitzschia frustulum*	4.42 ± 18.89	44
NDIS	*Nitzschia dissipata*	4.16 ± 11.66	64
CPED	*Cocconeis pediculus*	4.02 ± 15.3	72

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Code	Diatom taxa	Mean ± sd	n
GLAT	*Gomphonema lateripunctatum*	3.72 ± 14	33
ENMI	*Encyonema minutum*	3.48 ± 10.88	59
CNTH	*Cocconeis neothumensis*	3.47 ± 9.38	52
ENCM	*Encyonopsis microcephala*	3.45 ± 10.94	44
NINC	*Nitzschia inconspicua*	3.43 ± 15.47	53
PLFR	*Planothidium frequentissimum*	3.29 ± 11.27	66
GPAR	*Gomphonema parvulum*	3.25 ± 11.94	49
GCLE	*Gomphonema clevei*	3.15 ± 34.12	3
ADSU	*Achnanthidium subatomus*	3.11 ± 14.43	24
ADEU	*Achnanthidium eutrophilum*	3.01 ± 11.19	48
GRHB	*Gomphonema rhombicum*	2.86 ± 21.52	8
GAGV	*Gomphonema angustivalva*	2.81 ± 15.13	38
ESLE	*Encyonema silesiacum*	2.72 ± 8.25	64
EOMI	*Eolimna minima*	2.69 ± 7.66	57
MPMI	*Mayamaea permitis*	2.14 ± 13.41	24
DTEN	*Denticula tenuis*	2.06 ± 13.7	34
AMPS	*Amphora indistincta*	1.86 ± 7.8	41
MVAR	*Melosira varians*	1.82 ± 13.73	31
CEUO	*Cocconeis euglyptoides*	1.82 ± 10.71	34
NIPM	*Nitzschia perminuta*	1.76 ± 23.45	1
ADMF	*Achnanthidium affine*	1.76 ± 8.08	40
GOMP	*Gomphonema sp.*	1.64 ± 8.59	31
CPLA	*Cocconeis placentula*	1.54 ± 7.5	21
ADAT	*Achnanthidium atomus*	1.51 ± 5.78	40
GTER	*Gomphonema tergestinum*	1.47 ± 6.77	36
UULN	*Ulnaria ulna*	1.42 ± 5	47
NCPR	*Navicula capitatoradiata*	1.25 ± 4.79	38
BNEO	*Brachysira neoexilis*	1.22 ± 10.77	11
ESUM	*Encyonopsis subminuta*	1.22 ± 4.03	36
NRCH	*Navicula reichardtiana*	1.2 ± 3.08	53
GOLI	*Gomphonema olivaceum*	1.19 ± 3.28	48
NPAL	*Nitzschia palea*	1.18 ± 3.57	43
FDEL	*Fragilaria delicatissima*	1.17 ± 12.78	8
ADSB	*Achnanthidium straubianum*	1.16 ± 5.2	34
DMON	*Diatoma moniliformis*	1.09 ± 5.99	30
ESBM	*Eolimna subminuscula*	1 ± 3.38	35
CEXF	*Cymbella excisiformis*	0.98 ± 4.96	16
NPAE	*Nitzschia paleacea*	0.98 ± 6.92	14

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Table S3.3 – *Continued from previous page*

Code	**Diatom taxa**	**Mean ± sd**	**n**
DVUL	*Diatoma vulgaris*	0.97 ± 3.26	29
RUNI	*Reimeria uniseriata*	0.94 ± 3.74	37
DOCU	*Diploneis oculata*	0.91 ± 7.59	9
NGRE	*Navicula gregaria*	0.89 ± 3.05	32
ACHD	*Achnanthidium sp.*	0.88 ± 3.82	15
NAMP	*Nitzschia amphibia*	0.85 ± 3.95	20
FRUM	*Fragilaria rumpens*	0.84 ± 3	23
ADSA	*Achnanthidium saprophilum*	0.82 ± 2.81	27
CMEN	*Cyclotella meneghiniana*	0.81 ± 6.08	19
ADCA	*Achnanthidium caledonicum*	0.8 ± 5.26	16
CAFF	*Cymbella affinis*	0.78 ± 4.46	9
NLAN	*Navicula lanceolata*	0.77 ± 4.63	17
COCS	*Cocconeis sp.*	0.75 ± 5.12	8
DDEL	*Delicata delicatula*	0.75 ± 4.17	23
FGRA	*Fragilaria gracilis*	0.72 ± 3.37	20
ADGL	*Achnanthidium gracillimum*	0.7 ± 8.38	4
FCVA	*Fragilaria capucina var. vaucheriae*	0.7 ± 4.26	18
GANT	*Gomphonema angustum*	0.69 ± 5.02	6
PTLA	*Planothidium lanceolatum*	0.68 ± 2.43	31
NRAD	*Navicula radiosa*	0.66 ± 5.61	11
FPEM	*Fragilaria perminuta*	0.64 ± 3.61	16
NANT	*Navicula antonii*	0.62 ± 2.33	34
ENCY	*Encyonopsis sp.*	0.61 ± 5.51	8
DEHR	*Diatoma ehrenbergii*	0.55 ± 3.42	13
DITE	*Diatoma tenuis*	0.54 ± 4.91	6
MAPE	*Mayamaea atomus var. permitis*	0.53 ± 2.31	21
NCRY	*Navicula cryptocephala*	0.52 ± 2.77	14
FAUT	*Fragilaria austriaca*	0.51 ± 2.96	15
UUAC	*Ulnaria acus*	0.49 ± 2.61	15
CDTG	*Cyclotella distinguenda*	0.45 ± 3.69	10
CPPV	*Cymbella perparva*	0.44 ± 4.47	2
NVEN	*Navicula veneta*	0.43 ± 1.58	30
NCTO	*Navicula cryptotenelloides*	0.39 ± 2.53	16
FCAP	*Fragilaria capucina*	0.38 ± 3.12	8
CLCT	*Caloneis lancettula*	0.36 ± 1.34	24

Table S3.4 Mean abundances, standard deviation, and number of occurrences (n) of fish species across the selected sampling sites (n=264).

Fish taxa	Mean ± sd	n
Salmo trutta	39.39 ± 54.27	219
Phoxinus bigerri	37.91 ± 104.93	109
Parachondrostoma miegii	8.84 ± 39.63	35
Salmo salar	7.3 ± 33.81	63
Anguilla anguilla	5.62 ± 13.19	102
Luciobarbus graellsii	4.99 ± 20.48	33
Barbus haasi	3.83 ± 32.82	24
Gobio gobio	3.79 ± 19.01	25
Achondrostoma arcasii	3.01 ± 17.57	23
Barbatula barbatula	2.72 ± 11.42	36
Squalius valentinus	2.57 ± 38.6	2
Pseudochondrostoma willkommii	2.07 ± 16.43	10
Luciobarbus sclateri	1.86 ± 13.17	12
Squalius pyrenaicus	1.35 ± 8.83	12
Barbus meridionalis	1.04 ± 6.3	8
Pseudochondrostoma duriense	1.03 ± 6.51	16
Cobitis calderoni	1.03 ± 12	4
Squalius malacitanus	1 ± 10.95	3
Squalius alburnoides	0.52 ± 5.2	4
Cobitis paludica	0.38 ± 4.39	3
Petromyzon marinus	0.31 ± 2.07	15
Squalius carolitertii	0.3 ± 3.25	3
Squalius laietanus	0.24 ± 2.62	4
Lampetra planeri	0.2 ± 2.39	2
Alburnus alburnus	0.17 ± 2.15	4
Luciobarbus guiraonis	0.15 ± 2.34	2
Iberochondrostoma lemmingii	0.12 ± 1.91	1
Lepomis gibbosus	0.11 ± 1.56	2
Gasterosteus aculeatus	0.09 ± 1.3	2
Luciobarbus bocagei	0.09 ± 1.42	1
Parachondrostoma arrigonis	0.08 ± 0.91	2
Carassius auratus	0.05 ± 0.86	1
Oncorhynchus mykiss	0.04 ± 0.4	4

Continued on next page

Table S3.4 – *Continued from previous page*

Fish taxa	**Mean ± sd**	**n**
Platichthys flesus	0.04 ± 0.47	3
Cottus aturi	0.02 ± 0.25	2
Salaria fluviatilis	0.01 ± 0.17	2
Tinca tinca	0.01 ± 0.11	3
Alosa alosa	0.01 ± 0.13	2

3.5.1 Selection of the spatial weighting matrices (SWM) and Moran's eigenvector maps (MEMs)

The spatial weighting matrix (SWM) selection was carried out using used an optimisation method proposed by Bauman et al. (2018b). We used only graph-based SWM methods, as our sampling design is highly irregular. The distance-based connectivity methods, which are often used in ecological studies, can lead to large truncation values as they allow all sites to remain connected in the map, losing fine spatial structures (Borcard et al., 2018).

With the function `listw.select` from the `adespatial` package (Dray et al., 2020), we tested three graph-based connectivity schemes: Gabriel graph, relative neighbour graph, and minimum spanning tree. The connectivity schemes are combined with two weighting matrices: binary (i.e. no weights attributed to the connections) or linearly (i.e. decreasing as a function of sites distance), defined by $1 - (D/d_{max})$, where D is the Euclidean distance between two sites and d_{max} is the maximum Euclidean among all site pairs.

In the optimisation process, the p-value was corrected for multiple tests using the Sidak correction (Šidák, 1967) to avoid inflated type I error rates, so the significance threshold decreases with an increasing number of SWM candidates. The SWM selection was based on the maximisation of the adjusted R^2 (Table S3.5), calculated for all positive MEMs in each SWM. We used all positive MEMs, since a MEM subset (traditionally selected via forward selection in most ecological studies) does not successfully remove spatial autocorrelation from the model residuals, generating inflated estimates in variation partitioning (Clappe et al., 2018; Peres-Neto and Legendre, 2010).

For both diatoms and fish, the SWM yielding the highest adjusted R^2 was the relative neighbour graph, with the linear weights for community composition and binary for functional traits. Then, the positive MEMs generated based on the selected

Table S3.5 Six SWM tested in the optimisation method and the adjusted R^2 values obtained with the positive MEM model in each case. Values in bold correspond to the highest values.

SWM	Diatoms		Fish	
	C-TX	**C-FT**	**C-TX**	**C-FT**
Gabriel graph - Binary	0.239	0.339	0.510	0.450
Gabriel graph - Linear	0.241	0.350	0.515	0.444
Relative neighbour graph - Binary	0.249	**0.367**	0.529	**0.449**
Relative neighbour graph - Linear	**0.250**	0.359	**0.529**	0.449
Minimum spanning tree - Binary	0.235	0.330	0.517	0.446
Minimum spanning tree - Linear	0.238	0.324	0.521	0.447

SWMs (relative neighbour graph, with linear or binary weights) were used as spatial variables in the variation partitioning test with MSR correction (Figure S3.2).

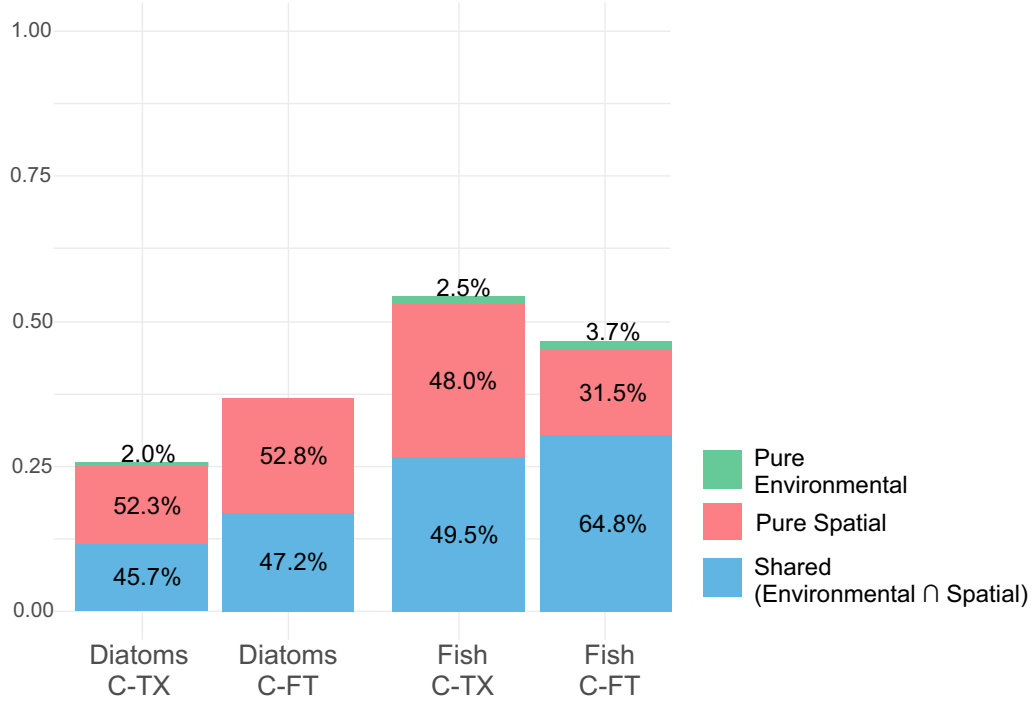


Figure S3.2 Variation partitioning after MSR correction of environmental and spatial components explaining diatoms and fish community composition and functional traits. Percentages indicate the fractions' contribution in the total explained variation.

Chapter 4

Effects of irrigation dams on riverine biota in mountain streams

Abstract

Mountain streams harbour unique biodiversity and provide essential ecosystem services. Yet, these freshwater ecosystems have been facing numerous threats, such as the construction of dams and land use changes, leading to rapid habitat degradation, water pollution, and biodiversity loss. In this study, we assess the effect of irrigation dams on mountain riverine biota using traditional biotic indices and trait-based approaches. We selected diatom and macroinvertebrate communities surveyed between 2015 and 2017 in mountain streams located in northern Spain (Cantabrian Cordillera, Iberian System, and Pyrenees) under natural or altered flow conditions (i.e. located downstream of irrigation dams). We identified hydrological and biological changes associated to the presence of dams, the mountain range, or the interaction between these two factors. Summer flows (e.g. magnitude of July, August, and September flows), frequency of high flow events, and the timing of minimum annual flows were significantly affected by irrigation dams, independently of the region. Winter flows, the magnitude of high flow extremes, and the number of flow rises and falls varied with the presence of dam and mountain range, and the flow pulses frequency and duration depended on the mountain range only. In the Cantabrian Cordillera, a region with relatively large reservoirs (>150 hm^3), impacted sites showed a marked inversion of the seasonal flow patterns (i.e. increases in summer flows but reduced winter flows). In the other mountain ranges, reservoirs

had smaller storage volumes and multiple purposes, causing significant variations in the flow change frequency only. Diatom traits, taxonomic richness, diversity, and IPS score varied with the presence of dam and mountain range; macroinvertebrate traits and biotic indices did not respond significantly to flow regulation. It suggests that diatom communities might be more sensitive to hydrological alteration, while macroinvertebrates might be more influenced by spatial processes (e.g. dispersal) and biogeography, overriding the dam-related impacts. Our results highlight the relevance of exploring pre-existing datasets to understand the effects of dams on riverine ecosystems, as well as to support future conservation and biomonitoring strategies addressing river regulation impacts.

4.1 Introduction

Mountain streams provide a unique set of habitat conditions in the river network (i.e. fast water velocities and low temperatures) and harbour an important portion of the global biodiversity (Rahbek et al., 2019). Their high runoff from rainfall and the delayed releases of water stored as snow or lake reserves characterise mountains as "water towers", providing lowlands with relatively constant essential water resources and ecosystem services (Immerzeel et al., 2020; Viviroli and Weingartner, 2008). Population dependence on mountain water runoff is expected to grow steadily, reaching 1.5 billion people critically relying on these water resources by 2050 (Viviroli et al., 2020). At the same time, due to the narrow niches of endemic species, mountain environments are extremely vulnerable to global change. As elevation can amplify global warming effects (Christmann and Oliveras, 2020; Grabherr et al., 2001), even small environmental changes can cause significant ecological impacts, such as the reductions of glaciers and the ice-melt inputs into mountain rivers (Fell et al., 2017). Thus, mountain rivers might experience more rapid climate change effects than their lowland counterparts (Pepin et al., 2015).

An extensive number of mountain rivers have been affected by dams and other water infrastructures, e.g. in the Pyrenees (López-Moreno et al., 2008), in the Alps (Spitale et al., 2015), and in the Colorado Front Range (Wohl, 2006). Apart from the hydropower potential, which is extensively exploited for energy production, mountain dams store and build up water reserves during seasons of heavy snow melt for lowland irrigation and domestic water uses in low flow periods, as well as supply water to mountain lakes used for tourism and fishing (Consoli et al., 2021; Marnezy, 2008). These infrastructures tend to homogenise flow dynamics (i.e. reducing peak flows and increasing minimum flows; Moyle and Mount, 2007), alter the downstream water temperature, the nutrient and sediment fluxes (Maavara et al.,

2020; Olden and Naiman, 2010), and limit the habitat connectivity (Grill et al., 2019). It leads to pervasive effects on riverine biodiversity and functioning, e.g. reduction of macroinvertebrate richness (Kennedy and Turner, 2011) and the salmon populations downstream of dams (Moore et al., 2011). Therefore, identifying, monitoring, and managing these impacts on riverine ecosystems while securing human water needs for socioeconomic development has become a major challenge for water resource managers, especially in highly vulnerable regions (e.g. wetlands and mountains; Pang et al., 2018).

Diatoms and macroinvertebrates have been widely used as environmental indicators due to their easy sampling, widespread occurrence, diversity, and reliable indication of anthropogenic impacts (Bonada et al., 2006; Resh, 2008) and are among the biological quality elements recommended to monitor and assess water bodies' ecological state within the European Water Framework Directive (WFD; European Commission, 2000). Their taxonomy-based biotic indices are also extensively employed to measure ecological responses to nutrient enrichment and organic pollution; however, the effects of flow modification are rarely addressed (Birk et al., 2012). In addition, the taxonomic-based metrics may be subject to significant biogeographic and evolutionary constraints (Soininen et al., 2016), reducing their broad geographic applicability (Dolédec et al., 1999). Thus, species traits (i.e. organism's biological and ecological characteristics) have been increasingly used in ecological research (e.g. Brown et al., 2019; White et al., 2017a; Wu et al., 2019b) to assess stressor effects in lotic ecosystems and to develop mechanistic trait-stressor links valid across broad geographical scales (Culp et al., 2011; Statzner and Bêche, 2010).

In this context, the data originally collected for water quality assessment (e.g. WFD surveys), including biological communities and physico-chemical habitat conditions, may be used to address other eco-hydromorphological issues in rivers, such as the ecological effects of dams and other anthropogenic stressors (Feeley et al., 2020; Krajenbrink et al., 2019; Vaughan and Ormerod, 2010). To do so, an efficient monitoring network is essential (Arrighi et al., 2021) since the actual effects of hydrological alteration may be underestimated by inadequate spatial (e.g. lack of gauges immediately downstream of dams; Peñas et al., 2016) and temporal coverages (e.g. biological sampling not covering inter- and intra-annual hydrological variability; Munné and Prat, 2011).

In this exploratory study, our main objective was to assess the effects of dams primarily used for irrigation (although they might include other minor uses such as water supply and hydropower) on the river flow regime, water quality characteristics, and riverine biota (i.e. diatom and macroinvertebrate communities). We selected

sites in streams under natural and regulated flow regimes (i.e. downstream of irrigation dams) and, to reduce the effects of geographical distance on generating differences among riverine communities (Chapter 3), our site selection for impacted and control streams was clustered around three mountain ranges in northern Spain: the Cantabrian Cordillera, the Iberian System, and the Pyrenees. We hypothesised that (1) the presence of dams would cause hydrological and water physico-chemical alterations; however, (2) part of these responses would also be dependent on the "mountain factor", i.e. the local conditions present in each mountain range (e.g. climate, altitude, geology, land use and land cover, and dams' specific characteristics). (3) Diatoms and macroinvertebrates would respond differently to the presence of dams due to their specific characteristics (e.g. body size, dispersal abilities, and life history patterns) and, (4) in relation to taxonomic biotic indices, the trait-based approaches would better capture the influence of dams on biological communities.

4.2 Methodology

4.2.1 Study area and site selection

Our study area covered streams located in three mountain ranges in northern Spain: the Cantabrian Cordillera, the Iberian System, and the Pyrenees (Figure 4.1a). These mountains constitute the natural geographical limits of the Ebro river catchment and present a significant number of structures to attend to water and energy demands (e.g. dams and reservoirs for hydroelectric power generation, irrigation, urban water supply, and recreation; López-Moreno et al., 2008), making them an interesting case study. Most dams in the Pyrenees, for example, were constructed between the 1950s and the 1980s to supply water demands in the Mediterranean region (i.e. irrigation and hydropower production; López-Moreno et al., 2008). In the Cantabrian Cordillera, besides several small hydroelectric power plants, important reservoirs (e.g. Ebro, Aguilar de Campoo, Riaño) supply water for agricultural and urban uses. Reservoirs in the Iberian System, in general, have smaller capacities in relation to the other mountain ranges and provide water for irrigation, urban uses, and hydropower generation.

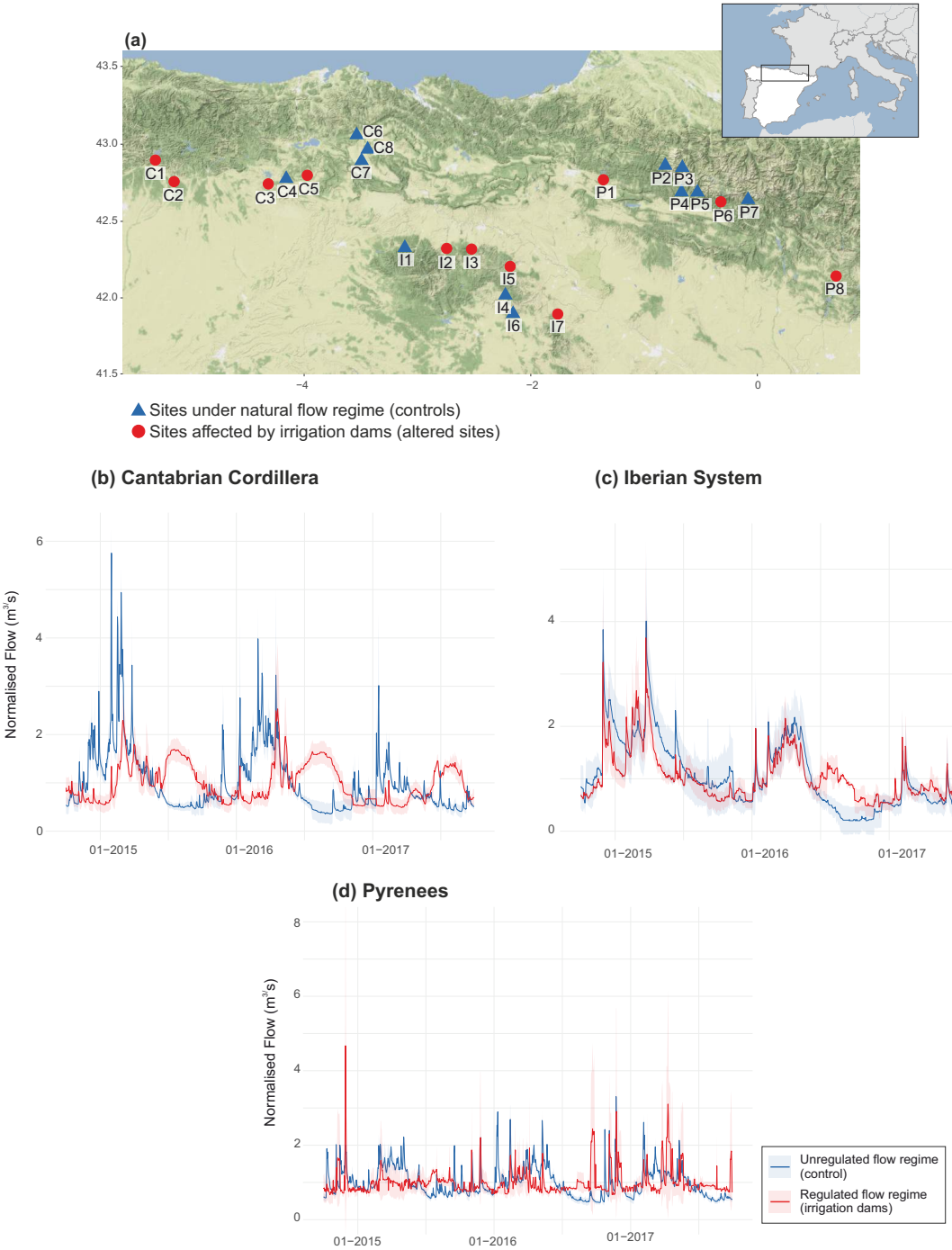


Figure 4.1 (a) Location of the 23 studied streams: controls (n=12) and sites affected by irrigation dams (n=11). Mean normalised daily discharge between 2015 and 2017 in controls and sites affected by irrigation dams in (b) the Cantabrian Cordillera, (c) the Iberian System, and (d) the Pyrenees. Shaded areas represent 1SD confidence intervals. See Table S4.1 in Supplementary Material for information on sampling sites and reservoirs.

Table 4.1 Catchment-scale environmental variables (describing topography, climate, the fractions occupied by land use and land cover, and lithology) in controls and sites affected by dams in each region. Values refer to the catchment mean ± sd upstream of the sampling site.

	Cantabrian Cordillera		**Iberian System**		**Pyrenees**	
	Control (n=4)	**Altered (n=4)**	**Control (n=3)**	**Altered (n=4)**	**Control (n=5)**	**Altered (n=3)**
Catchment size (km^2)	287.1 ± 166.6	640.4 ± 293.4	76.9 ± 29.6	421 ± 160.1	86.1 ± 81.5	581.5 ± 382.2
Elevation above sea level (m)	642.2 ± 121.3	859.9 ± 132.9	992.1 ± 75	586 ± 62.9	989.9 ± 179.9	609.7 ± 185.1
Slope in segment (0 to 1)	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Mean annual precipitation (mm)	806.6 ± 71.4	1053.2 ± 241.5	828.3 ± 318.6	738.5 ± 154.1	1538.5 ± 170.6	1378.1 ± 268.8
Mean annual temperature (°C)	10.1 ± 0.8	8.6 ± 1.1	8.9 ± 0.2	9.5 ± 0.4	8.1 ± 1.3	9.8 ± 1.1
Mean annual evapotranspiration (mm)	522 ± 26.1	531.3 ± 34.6	440.7 ± 52	433.4 ± 30.9	426.9 ± 31.6	507.5 ± 59.4
Fraction occupied by urbanisation	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Agriculture	0.3 ± 0.1	0 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	0 ± 0	0 ± 0
Pastures	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.4 ± 0.2	0.3 ± 0.1
Broadleaf forests	0.2 ± 0.1	0.2 ± 0	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.2
Coniferous forests	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0
Plantations	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0
Heathland and shrubs	0.1 ± 0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0 ± 0	0.2 ± 0.1
Denuded areas	0.1 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0.2 ± 0.1	0.1 ± 0.1
Fraction occupied by calcareous rocks	0.9 ± 0.1	0.4 ± 0.3	0.7 ± 0.5	0.7 ± 0.3	0.6 ± 0.3	0.4 ± 0.2
Siliceous rocks	0 ± 0	0.1 ± 0.2	0.3 ± 0.5	0.1 ± 0.2	0 ± 0	0 ± 0
Conglomerate rocks	0.1 ± 0.1	0.4 ± 0.2	0 ± 0	0.1 ± 0.1	0.4 ± 0.3	0.2 ± 0.1
Sandstone	0 ± 0	0 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0.3 ± 0.2
Sedimentary rocks	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Volcanic rocks	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0.1

Our sampling site selection was restricted to sites complying with the following requirements: (1) river reaches classified as "good" or higher ecological status according to the River Basin Management Plans 2015-2021 (BOE, 2005) within the European WFD; (2) river reaches with available hydrological and water physico-chemical data; and (3) at least one survey on biological communities (diatoms or macroinvertebrates) between June and October 2015, 2016, or 2017. These criteria allowed to reduce the environmental variability and the effect of other anthropogenic stressors, such as water pollution and nutrient enrichment associated to human activities (e.g. extensive urban or agricultural land uses). In total, we selected 23 sampling sites (Figure 4.1 and Table 4.1), from which 11 were affected by dams primarily used for irrigation (i.e. altered flow regime, see Table S4.1 in Supplementary Material) and 12 were under natural flow conditions (i.e. control sites).

River gauge stations provided by the Centre for Hydrological Studies of the Spanish Ministry for Ecological Transition (CEDEX, 2022) located nearby each sampling site recorded the daily river flow discharge. The normalised daily flow series (from 2015 to 2017) was then used to generate 35 hydrological indices (HIs; Table A.1 in Appendix A) characterising the flow regime components (magnitude, frequency, duration, timing, and rate of change).

4.2.2 Biological surveys and taxonomic-based biotic indices

Diatom and macroinvertebrate communities were surveyed annually by regional water agencies as part of the water bodies' ecological status assessment within the European WFD. This biological information was then compiled by the Spanish Ministry for the Ecological Transition in the national database NABIA (Spanish Royal Decree 817/2015 on Water Policy; BOE, 2005). Streams surveyed between June and October 2015, 2016, and 2017 were selected for this study and, in some of these sites, surveys occurred in consecutive years and covered both biological communities. In combination with the biological sampling, water temperature (T_{WATER}, °C), pH, dissolved oxygen (DO, mg.L^{-1}), electrical conductivity (μS.cm^{-1}), nitrite (NO_2^-; µg N.L^{-1}), nitrate (NO_3^-; μg N.L^{-1}), ammonium (NH_4^+; μg N.L^{-1}), and phosphate (PO_4^{3-}; μg P.L^{-1}) concentrations were determined using standard protocols (UNE-EN ISO 15681-2:2005 for phosphates, UNE-EN ISO 13395:1997 for nitrite and nitrates, UNE-EN ISO 11732:2005 for ammonium).

Diatom communities were surveyed following UNE-EN 13946:2014 (CEN, 2014a). Five to ten cobbles were randomly selected from the benthos (circa 100 cm^2 of exposed surface area) and the upper part of the substratum was scrubbed with a dishwasher brush. The material was decanted in a sample bottle and preserved using

formaldehyde. From each sample, 400 diatom valves were identified in laboratory using a microscope (1000× magnification) at the lowest feasible taxonomic level, according to the standard procedure UNE-EN 14407:2014 (CEN, 2014b). In total, 56 diatom communities were collected in 21 sampling sites (see Table S4.1 in Supplementary Material).

Benthic macroinvertebrate surveys followed the standard procedure UNE-EN 16150:2012 (CEN, 2012). The sampling was distributed among the different habitat types present along a 100-m reach. Using a kick hand net (0.1 m^2 and 0.5 mm mesh size), community samples were collected and preserved in 96% ethanol. The identification in the laboratory was mainly at the family level, except for Hydracarina, Nematoda, Ostracoda, Copepoda, and Oligochaeta. In total, 51 macroinvertebrate communities were collected in 21 sampling sites (see Table S4.1 in Supplementary Material).

We eliminated rare taxa (those representing less than 2% of the total sampled individuals when considering the total number of occurrences; Lavoie et al., 2009). In sites surveyed in multiple years, the abundance of taxa was averaged to obtain a site-specific assemblage structure and to reduce interannual variation, following Paavola et al. (2003) and Filker et al. (2016), resulting in 21 averaged communities for diatoms and 21 for macroinvertebrates. Likewise, for each site-specific assemblage, the average physico-chemical characteristics were calculated.

In each averaged biological community, taxa richness and Shannon-Wiener diversity index were computed using the R package `vegan` (Oksanen et al., 2019). Additionally, for diatom communities, we computed the Specific Polluosensitivity Index (IPS; CEMAGREF, 1982; MAGRAMA, 2013). For macroinvertebrates, the Iberian Average Score per Taxon (IASPT) and the Lotic invertebrate Index for Flow Evaluation (LIFE; Extence et al., 1999) were computed using the *R* package `biomonitoR` (Laini et al., 2019).

Diatom species were assigned to ecological guilds, life forms, and cell size (biovolume; Table B.1 in Appendix B), following Passy (2007) and Rimet and Bouchez (2012). In macroinvertebrate samples, taxa were classified into 11 biological traits for European macroinvertebrate genera (Schmidt-Kloiber and Hering, 2015; Tachet et al., 2010). The traits (Table B.2 in Appendix B) described the organisms' life history, morphology, food source, feeding habits, respiration, reproduction, among other characteristics using a fuzzy coding approach. Since most of the taxa were identified at the family level but traits are reported at the genus level, each trait affinity was averaged and re-scaled to this lower taxonomic resolution using affinities of all genera computed within a family. Although the use of family-level identification can result in a loss of ecological information, previous studies (Dolédec et al., 2000;

García-Roger et al., 2014; Gayraud et al., 2003) reported minor impacts on the functional description of macroinvertebrate communities.

4.2.3 Data Analysis

We used a two-way analysis of variance (ANOVA; factors: dam and mountain range) to test (a) the general effects of the presence of irrigation dams (control vs. altered sites), (b) the general effects of mountain ranges (Cantabrian Cordillera, Iberian System, and Pyrenees), and (c) the possible interactions between the presence of dams and the mountain range on the HIs, water physicochemical characteristics, and biotic indices. The tests were calculated with type III sums of squares due to the unequal sample sizes in control and altered sites among mountain ranges (Quinn and Keough, 2002). The homogeneity of variances was checked using Levene's tests and variables were log-transformed when needed. Factors detected to be significant ($p \leq 0.05$) were further analysed with Bonferroni multiple comparison tests.

We also used Spearman's rank correlations to identify statistically significant relationships ($p \leq 0.05$) among diatom and macroinvertebrate biotic indices, HIs, and physico-chemical characteristics.

The relationships between organisms' traits and environmental descriptors were investigated with the RLQ and fourth-corner analyses using the R packages `ade4` (Dray and Dufour, 2007) and `adespatial` (Dray et al., 2020). The RLQ analysis (Dolédec et al., 1996), an exploratory ordination method, combines three tables: table R ($n \times m$) containing the measurement of m environmental variables at n sites; table L ($n \times p$) with the abundance of p taxa at n sites; and table Q ($p \times s$) describing s traits related to the same p taxa. The method generates a fourth matrix representing the cross-variance structure explained by environmental variables and functional traits, providing a broad overview of how they are associated (Brown et al., 2014; Thioulouse et al., 2018). The overall significance of the trait-environment relationship was then assessed with 9999 permutations using a two-step procedure (Dray et al., 2014), which simultaneously test the permutation of sites (Model 2: testing the null hypothesis that the species distribution is randomly attributed to sites, irrespective of the site characteristics) and species (Model 4: testing the null hypothesis that species are distributed irrespective of their traits) while controlling the type I error (ter Braak et al., 2012). Further, the fourth-corner analysis was used to identify significant individual trait-environment relationships. This method computes a matrix ($s \times m$) with one-to-one trait-environment correlations (Dray et al., 2014).

Prior to the analyses, physico-chemical variables were standardised (zero mean and unit standard deviation) to reduce the effects of different measurement units and to improve normality (Quinn and Keough, 2002). The statistical significance level for the ANOVA tests, Spearman's rank correlation, and RLQ and fourth corner analyses was set to $p \leq 0.05$. All the analyses were performed in the *R* environment (R Core Team, 2020).

4.3 Results

4.3.1 Dam effects on HIs and physico-chemical variables

The two-way ANOVA tests associated to the presence of dams only ("D" factor in Figure 4.2) identified significant changes in the summer (+M7,8,9), winter (-M12), and spring flows (-M3), the frequency of high flow events (-FRE3), and the timing of minimum flows (-JMin). These findings indicated that seasonal flow inversions (i.e. increased summer flows but reduced spring and winter flows) and less frequent high flow events were hydrological changes caused by dams independently of their geographical region.

In turn, when considering the mountain factor only ("M" factor in Figure 4.2), the number of high and low flow pulses within a year (nPulsesLow, nPulsesHigh) and their duration (MeanDPLow, MeanDPHigh) varied significantly. In the Pyrenees, the Bonferroni post hoc tests showed that the high and low flow pulses were significantly more frequent than in the Iberian System ($p<0.04$), but shorter in duration in comparison to other regions ($p<0.001$ for meanDPHigh in all cases; $p=0.006$ for meanDPLow in relation to the Iberian System). These findings indicated that the frequency and duration of flow pulses varied among mountain ranges independently of the presence of dams.

The interaction between the effects of dams and the mountain range ("D×M" in Figure 4.2) caused significant differences in the winter (M1,2) and early summer flows (M6), the magnitude of short-term high flow extremes (1,3,7HF), and the number of days with increasing and decreasing flows (nPos, nNeg). The Bonferroni post hoc tests revealed that most of the significant differences were found between control and altered sites in the Cantabrian Cordillera (p at least <0.015 for M1, M2, M6) and in the Pyrenees (p at least <0.045 for nPos and nNeg).

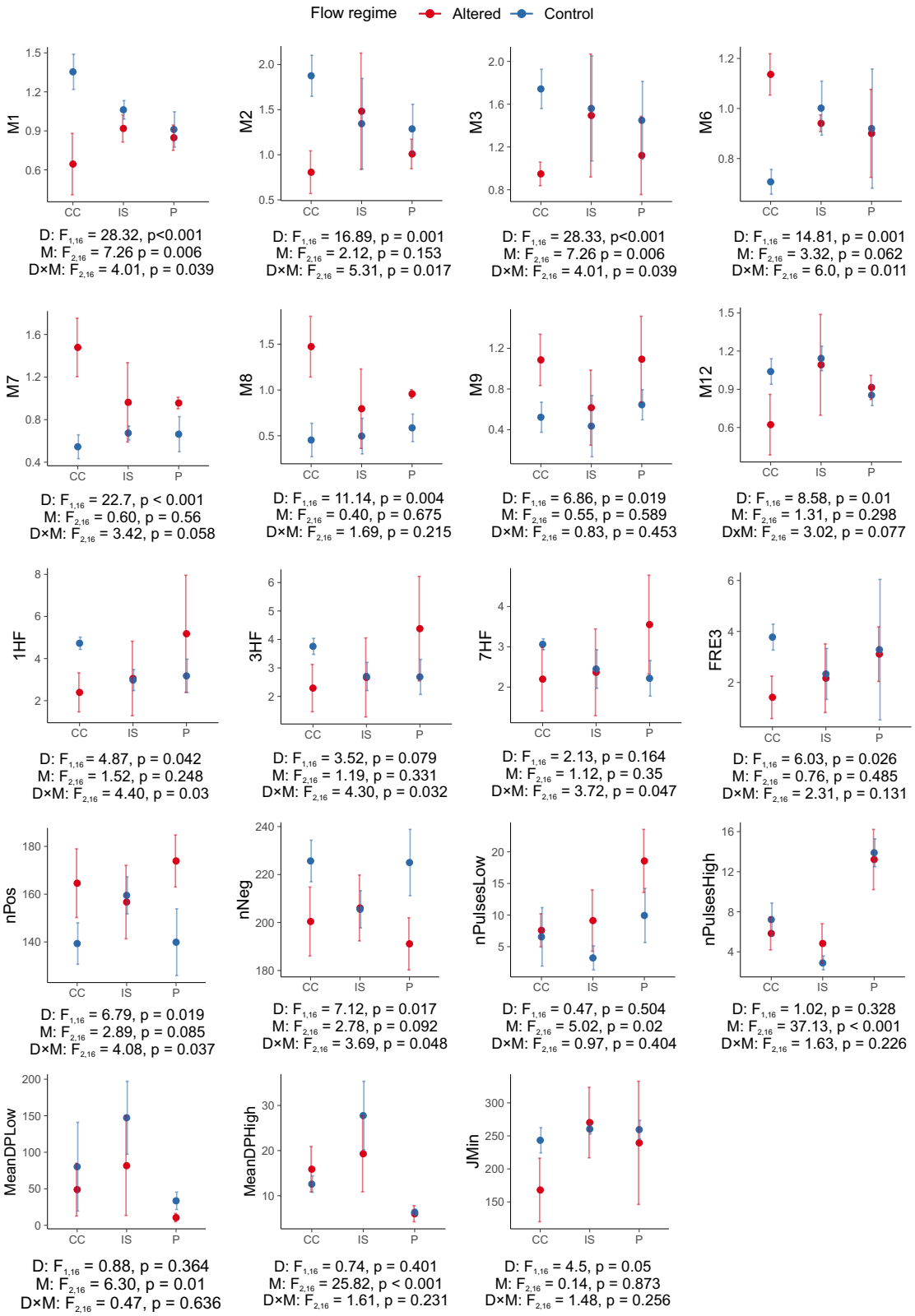


Figure 4.2 Interaction plots of hydrological indices with significant differences (see all variables in Figure S4.1 in Supplementary Material) identified in the two-way ANOVA tests in the Cantabrian Cordillera (CC), Iberian System (IS), and Pyrenees (P). "D" refers to the ANOVA test with flow regime (dam presence) as a factor, "M" to mountain range as a factor, and "D×M" as the interaction between these factors. Vertical bars represent the standard deviation.

In contrast to hydrology, physico-chemical characteristics did not show significant alterations caused by the presence of dams or their geographical location (Figure 4.3). Nitrate was the only variable that presented a marginally significant reduction downstream of dams (p=0.052).

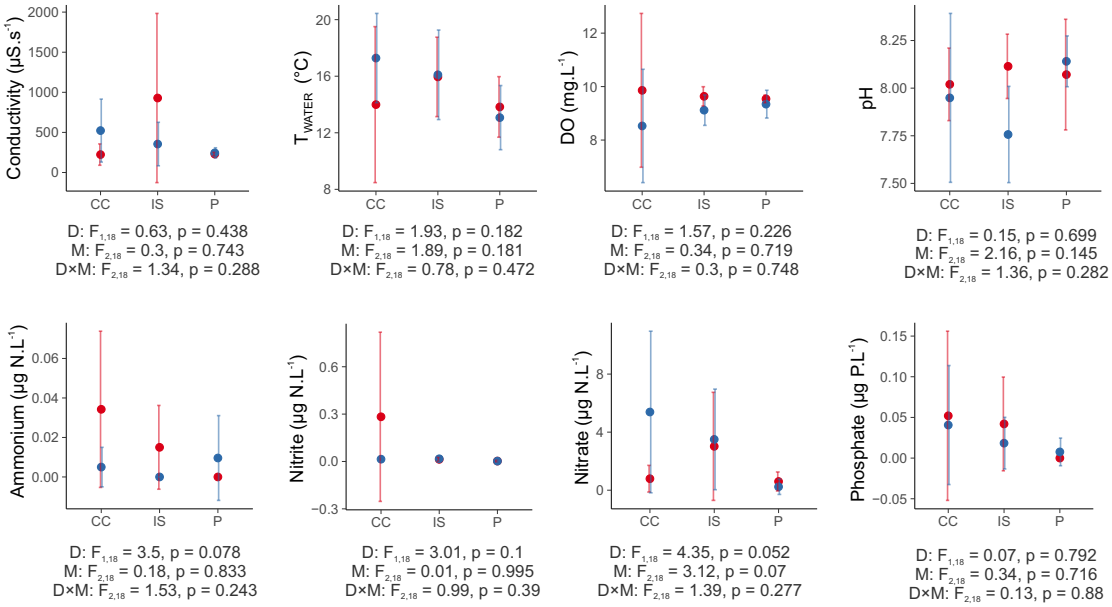


Figure 4.3 Interaction plots of physico-chemical variables using two-way ANOVA tests in the Cantabrian Cordillera (CC), Iberian System (IS), and Pyrenees (P). "D" refers to the ANOVA test with flow regime (dam presence) as a factor, "M" to mountain range as a factor, and "D×M" as the interaction between these factors. Vertical bars represent the standard deviation.

4.3.2 Effects of dams on taxonomy-based biotic indices

We retained 55 diatom species for the analyses (see Table S4.2 in Supplementary Material for taxa list). The most ubiquitous species were *Achnanthidium pyrenaicum* and *Achnanthidium minutissimum*. Sites under natural flow regime also showed higher mean abundances of *Gomphonema angustivalva* and *Achnanthidium subatomus*, while sites impacted by irrigation dams presented higher abundances of *Achnanthidium lineare* and *Cocconeis euglypta*.

The dam-mountain range interaction caused significant changes in the diatom taxonomic richness, Shannon-Wiener diversity index, and the IPS score (Figure 4.4a). The effect of dams varied among regions, with samples in the Iberian System (IS in Figure 4.4a) showing more clear changes associated to the dam presence: in altered sites, the richness and diversity increased, while the IPS score decreased in comparison to controls. However, the Bonferroni post hoc tests failed to detect significant differences. The Spearman's rank correlations (Figure S4.2a in Supplementary

Material) indicated that, in general, richness and Shannon-Wiener diversity were significantly linked to summer flows and water temperature.

In macroinvertebrate communities, a total of 43 taxa were retained for the analyses (Table S4.3 in Supplementary Material). Baetidae, Chironomidae, and Gammaridae were the most ubiquitous families. Control sites showed higher mean abundances of Chironomidae, Heptageniidae, and Leuctridae, while Simuliidae, Hydrobiidae, and Elmidae were more abundant in impacted sites.

In contrast to diatoms, the LIFE score (Figure 4.4b) was the only macroinvertebrate biotic index with significant changes associated to dams and the dam-mountain range interaction, although the Bonferroni post hoc tests did not detect significant differences between groups. On average, altered sites in the Cantabrian Cordillera scored higher than control sites, while in the other mountain ranges, scores in control and altered sites were similar. The Spearman's rank correlation (Figure S4.2b in Supplementary Material) indicated that the LIFE score was positively correlated to summer flows and the magnitude of low flow extremes but negatively correlated to winter flows and the frequency of high flow events.

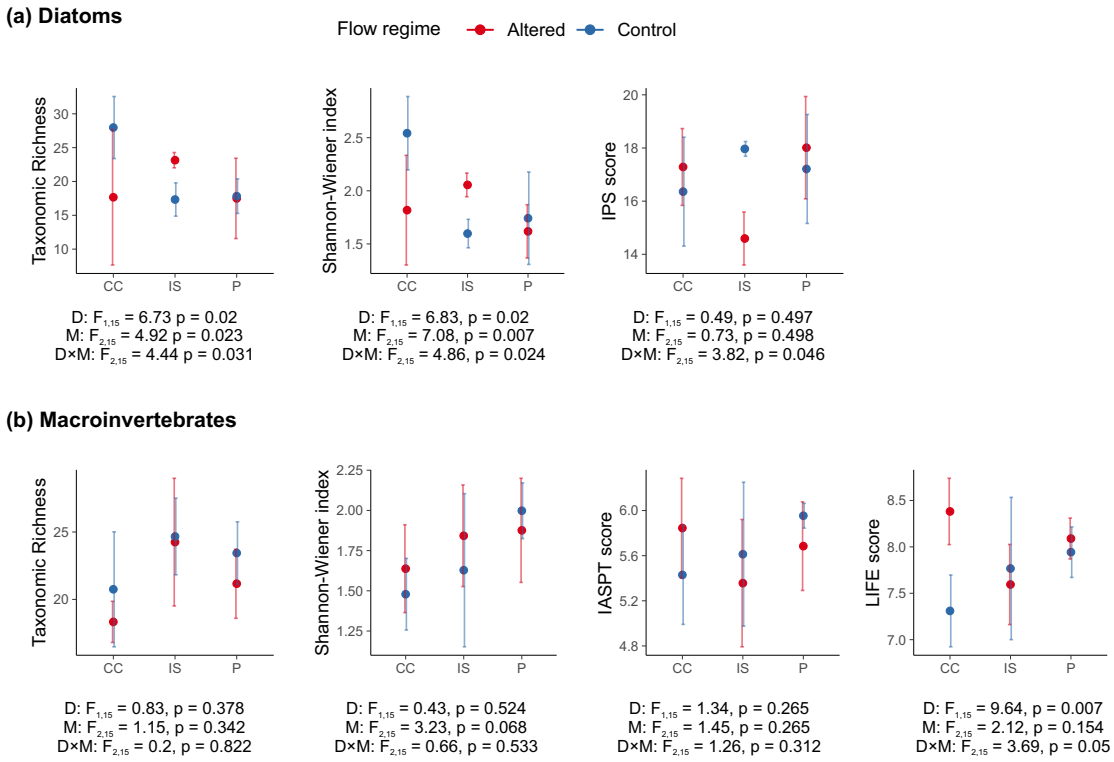


Figure 4.4 Interaction plots of biotic indices based on (a) diatom and (b) macroinvertebrate communities using two-way ANOVA tests in the Cantabrian Cordillera (CC), Iberian System (IS), and Pyrenees (P). "D" refers to the ANOVA test with flow regime (dam presence) as a factor, "M" to mountain range as a factor, and "D×M" as the interaction between these factors. Vertical bars represent the standard deviation.

4.3.3 Trait-based responses to dams in diatom communities

The overall RLQ permutation test on diatom communities showed a marginally significant trait-environment relationship (permutation across sites p=0.05, across species p=0.067). The first two axes of the RLQ analysis explained 85.7% of the cross-variance between diatom traits and HIs and physico-chemical conditions (Figure 4.5).

Along the first axis (67.7% of the total variance, Figure 4.5), the Cantabrian Cordillera and the Iberian System controls were concentrated on the negative side (left). Sites I5 and P8, which were affected by the multipurpose reservoirs Enciso and Escales, respectively (see Table S4.1 in Supplementary Material), were also clustered with controls. They were linked to more frequent and stronger high flow events (+FRE3,7, +1-90HF), February (+M2) and March (+M3) flows, and higher water conductivity, conditions that increased high profile and colonial diatoms while reducing the low profile guild (see fourth-corner analysis, Figure S4.3 in Supplementary Material). Most of these HIs showed to vary significantly with flow regulation as indicated in the two-way ANOVA tests (Figure 4.2) and, in the case of February flows and high flow extremes (1-7HF), with the interaction among flow regulation and the mountain range factors. In contrast, altered sites in the Cantabrian Cordillera and most of the altered sites in the Iberian System were linked to higher mean magnitude of spring (+M5) and autumn (+M10) flows and long-term low flow extremes (+7,30,90LF), favouring pioneer diatoms and reducing the high profile guild.

A further 17.9% of the total variance was explained by the second RLQ axis (Figure 4.5). Higher phosphate and ammonium concentrations, as well as stronger flow change rates (+meanPos, -meanNeg), observed especially in the C2 site, favoured adnate diatoms. In turn, pedunculate diatoms were linked to less strong flow change rates (+meanNeg) and lower nutrient concentrations.

4.3.4 Trait-based responses to dams in macroinvertebrate communities

The overall RLQ permutation test revealed a non-significant macroinvertebrate trait-environment relationship (permutation across sites p=0.318, across species p=0.47). The first two RLQ axes accounted for 70.3% of the total variance (Figure 4.6).

Along a gradient of flow regulation, sites under natural flow regime (i.e. controls) were more concentrated in the bottom part of the ordination, except for site I5,

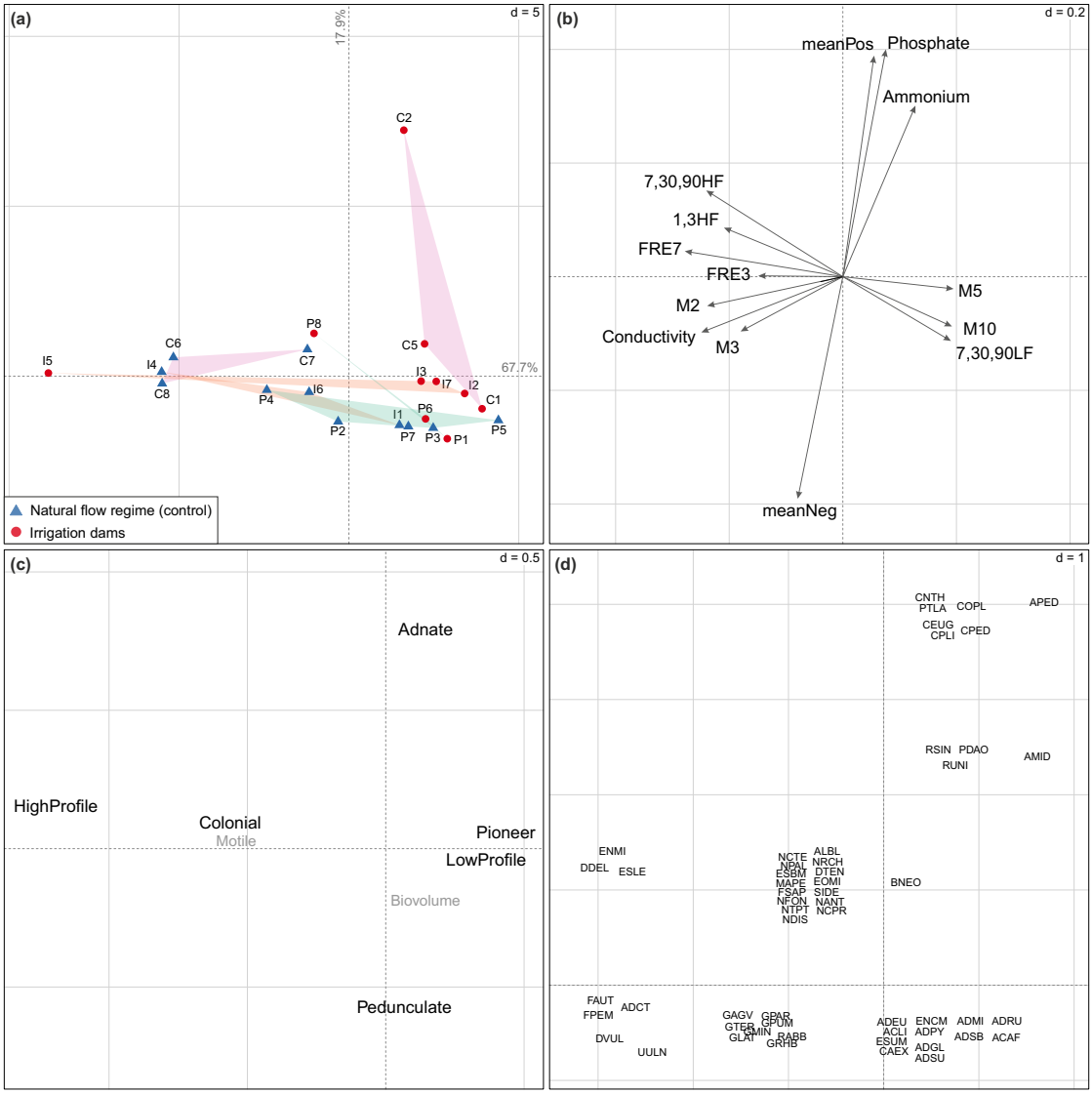


Figure 4.5 Ordinations showing the first two RLQ axes on diatom communities. (a) Site scores classified according to flow regulation and geographical region: Cantabrian Mountain Range (in pink), Iberian System (orange), and Pyrenees (green). (b) Coefficients for HIs (see Table A.1 in Appendix A for codes) and physico-chemical with strong links to traits (see fourth corner analysis in Figure S4.3). (c) Coefficients for traits (see Table B.1 in Appendix B for codes). Traits with no significant relationships are represented in grey (see fourth-corner analysis, Figure S4.3). (d) Coefficients for species (see Table S4.2 for codes). The "d" values give the grid size for scale comparison across the graphs.

which was affected by the reservoir Enciso (a multipurpose reservoir, see Table S4.1 in Supplementary Material). The Cantabrian Cordillera and Iberian System controls were mostly clustered in the bottom-left quadrant. These sites, which were located at lower altitudes than altered ones (see Table 4.1), were associated to increased frequency (+FRE7) and magnitude of high flow events (+30HF), winter (+M1,2) and spring (+M3,4) flows, later timing of minimum and maximum annual extremes (+JMin, +JMax), and higher water temperatures (+T_{WATER}). These conditions

favoured macroinvertebrates with small body sizes (<1cm, Small, e.g. Hydroptilidae), more than one reproductive cycle per year (Multivoltine, e.g. Sphaeriidae), deposit feeders, and fine detritus (FineDetritus, e.g. Ostracoda, Caenidae) and animal detritus (AnimalDetritus; e.g. Lymnaeidae) as food sources (see fourth-corner analysis, Figure S4.4 in Supplementary Material).

In turn, sites affected by irrigation dams were mostly concentrated in the top-right quadrant (except for control sites P5 and P7). These altered sites were associated to increased spring (+M5), summer (+M6,7), and autumn (+M10) flows and increased magnitude of short-term low flow extremes (+1,3LF). These conditions favoured macroinvertebrates with larger body sizes (>4 cm, Large, e.g. Pediciidae), one potential reproductive cycle per year (Univoltine), larval aquatic stages (Larva, e.g. Perlidae), aquatic (AquActive, e.g. Ephemerellidae) and aerial active (AerialActive, e.g. Heptageniidae) dispersions.

Following the same pattern observed in the diatom's RLQ ordination (Figure 4.5), macroinvertebrate communities in the Cantabrian Cordillera showed a more pronounced difference among control and impacted sites than the other mountain ranges. Along the second axis (23.1% of the total variance, Figure 4.6), the Cantabrian Cordillera impacted sites, which were marked by increased summer flows (+M6,7), showed to favour taxa with larger body sizes and larvae as aquatic stage. In contrast, controls, i.e. sites with increased winter flows (+M1,2) and higher water temperatures, favoured small-sized macroinvertebrates and detritus feeders.

A nutrient concentration and flow change gradient was observed along the first axis (47.2% of the total variance, Figure 4.6). Iberian System sites were more concentrated on the left side with increased nitrate concentration and longer low flow pulses, favouring macroinvertebrates with medium to large body sizes (MedLarge, e.g. Rhyacophilidae) and adult aquatic stage (Adult, e.g. Hydrobiidae, Gammaridae). In contrast, lower nitrate concentrations and more frequent and shorter flow pulses (+nPulsesHigh, -MeanDPLow) were more associated to Pyrenees' sites, favouring macroinvertebrates with medium body sizes (Medium, e.g. Heptageniidae), aquatic larvae, and aerial active dispersion.



Figure 4.6 Ordinations showing the first two RLQ axes on macroinvertebrate communities. (a) Site scores according to flow regulation and geographical region: Cantabrian Mountain Range (pink), Iberian System (orange), and Pyrenees (green). (b) Coefficients for HIs (see Table A.1 for codes) and physico-chemical variables with strong links to traits (see fourth corner analysis in Figure S4.4). (c) Coefficients for traits (see Table B.2 in Appendix B for codes). Traits with no significant relationships are represented in grey (see fourth-corner analysis, Figure S4.4). (d) Coefficients for species (see Table S4.3). The "d" values give the grid size for scale comparison across the graphs.

4.4 Discussion

In this study, we assessed the effects of irrigation dams on the riverine flow regime, water quality, and biota. Summer flows (e.g. magnitude of July, August, and September flows), frequency of high flow events, and the timing of minimum annual flows showed to be significantly affected by the presence of dams, independently of the local environmental conditions. Winter flows, magnitude of high flow extremes, and flow pulse changes, in contrast, were dependent on the local conditions (i.e. mountain range factor). Diatom communities (traits and taxonomic biotic indices) presented significant responses to dam impacts across the three mountain ranges; macroinvertebrates, on the other hand, showed less clear responses to hydrological and environmental conditions, as they might be more influenced by spatial-related processes (e.g. dispersal) and biogeography.

4.4.1 Abiotic changes associated to dams

We identified global alterations in the flow regime associated to the presence of irrigation dams in our dataset. In general, the magnitude of summer and autumn flows (i.e. HIs characterising the seasonal flow patterns), the frequency of high flow events, and the timing of minimum annual flows varied significantly with the presence of dams. These HIs seem to better indicate flow regulation effects caused by irrigation dams than other hydrological variables that may be under the effect of local conditions. In contrast, winter flows, high flow extremes, and the number of flow rises and falls showed to be significantly affected by the presence of dams and the geographical location (i.e. interaction among flow regulation and mountain range factors), and flow pulse changes were related to the mountain range factor (i.e. affected by local conditions only).

The Cantabrian Cordillera, when compared to other mountain ranges, displayed a pronounced inversion of the seasonal flow regime, with increased summer flows but lower winter flows, i.e. retention of winter flow peaks and release of summer flows to address seasonal water demands, as also reported in previous studies (Belmar et al., 2013; García de Jalón et al., 2019). The reservoirs in this region are predominantly operated to attend to agricultural water demands, and their relatively large capacity (e.g. Riaño, Ebro, Juan Benet, >150 hm^3 of storage volume; see Table S4.1 in Supplementary Material) contributes to the pronounced seasonal flow inversions.

In the other regions, the presence of dams was not associated to significant seasonal flow changes. In the Pyrenees, the number of flow rises within a year was significantly higher downstream of dams; in the Iberian System, no significant

changes in HIs were detected. This might be due to the substantially lower storage volumes of the reservoirs and their multiple purposes (e.g. Bubal, Lanuza, and Escales are also used for flood control and hydropower production; López-Moreno et al., 2003). Independently of the dam presence, the high and low flow pulses showed to be shorter and more frequent in the Pyrenees, especially in comparison to the Iberian System, which can be associated to the higher altitudes, slope, and increased precipitation found in Pyrenean sites. In addition, a slight reduction in the spring flows was observed in altered sites in the Iberian System and the Pyrenees streams (see Figure S4.1 in Supplementary Material), which could be related to the retained spring snowmelt flows occurring in these mountain areas (Dott et al., 2022). These findings highlight the differences in the local conditions and the dam characteristics among mountain ranges, which are reflected in the hydrological responses to flow regulation.

The physico-chemical variables did not show significant changes associated to irrigation dams or the mountain range. Although water temperature and nutrient concentration are reported to be strongly impacted by dams (Chandesris et al., 2019; Maavara et al., 2020; Olden and Naiman, 2010), the available physico-chemical data (spot measurements collected along with the annual biological surveys) might be insufficient to demonstrate the dam impact trends. Therefore, continuous water quality monitoring would be required to better understand the effects of irrigation dams on these variables (Vilmin et al., 2018).

4.4.2 Changes in biotic indices associated to dams and mountain ranges

The diatom biotic indices showed significant changes with the flow regulation-mountain range interaction. In the Iberian System, slightly higher taxonomic richness and diversity were detected in regulated sites, in agreement with previous studies (Krajenbrink et al., 2019; Smolar-Žvanut and Mikoš, 2014). It suggests that diatom communities might benefit from more stable flows in regulated sites, as these conditions favour the development of biofilm algae (Biggs and Smith, 2002; Biggs et al., 1998), and the reduction of grazers downstream of dams may also contribute to a more diverse community (Liess and Hillebrand, 2004). The IPS score, which was originally developed to target organic pollution (CEMAGREF, 1982), showed a reduction in flow-regulated sites in the Iberian System only. Since the index did not respond significantly to HIs in our study, it could be linked to local environmental factors (e.g. higher altitudes and higher siliceous geology in controls

in comparison to altered sites in the Iberian System) or other water quality factors not covered in our study.

The macroinvertebrates' taxonomic richness and the Shannon-Wiener diversity did not show significant responses to flow regulation. Although numerous studies indicate that dam construction decrease macroinvertebrate richness (Wu et al., 2019a), these biotic indices were weakly correlated to hydromorphological alterations in our study (see Spearman's rank correlation in Figure S4.2 in Supplementary Material), which was also seen elsewhere (Feld et al., 2014). Thus, the taxonomic replacement associated to hydrological alteration might not cause changes in these whole-community biotic indices, as ecologically similar species replace those lost with hydrological impacts (Feld et al., 2014). Similarly, the IASPT score did not respond to flow regulation, in line with previous research (Schneider and Petrin, 2017). This index is based on macroinvertebrate families' sensitivity to organic pollution and was originally designed to target water quality rather than other stressors (Friberg et al., 2011).

The LIFE index, a UK-based index designed to evaluate hydromorphological stress (Extence et al., 1999), failed to detect dam impacts downstream of irrigation dams as altered sites in the Cantabrian Cordillera scored higher than control sites. This divergence could be attributed to factors other than hydrology (e.g. water quality, channel morphology, and anthropogenic alteration of riverine habitats), which were not fully addressed in our study but may have a significant impact on the macroinvertebrate community structure and thus on the LIFE scores (Laini et al., 2018; Worrall et al., 2014). Differences in the biota hydraulic requirements between Spanish mountain streams and UK streams could also contribute to these results (Armanini et al., 2011; Laini et al., 2018). Furthermore, a long-term biological sampling scheme would be ideal to detect changes in macroinvertebrate communities and biotic indices (Vaughan and Ormerod, 2012).

4.4.3 Changes in trait-based communities associated to dams and mountain ranges

As hypothesised, the trait-based biological responses varied with the different flow regime patterns and mountain ranges. In the Cantabrian Cordillera, communities showed a clear segregation between control and impacted sites, which can be linked to the marked hydrological alteration identified in this region (i.e. seasonal flow inversion with increased summer flows and lower winter flows) causing shifts in the biological communities. In contrast, in the Iberian System and the Pyrenees, these shifts associated to seasonal flow inversions were less evident, and communities in

the altered sites I5 and P8 were similar to controls. Specifically in these sites, the multiple-purpose dams (used for irrigation, urban water supply, and hydropower generation) may cause different hydrological alteration patterns (e.g. changes in flow rise frequency). In addition, the distances between the sampling location and the dams were relatively large (>20km, with natural tributaries) in comparison to most altered sites (Table S4.1), which might have buffered the dam-related hydrological and biological impacts.

The diatom trait-based analysis indicated that control sites, in general, favoured the high profile guild and colonial life forms, i.e. traits associated to low flow disturbances (Passy, 2007). Dam-impacted sites, in turn, favoured pioneer and low profile diatoms, i.e. resistant traits associated to highly disturbed habitats (Passy, 2007; Tornés et al., 2015). Goldenberg-Vilar et al. (2021) reported that resistant traits (e.g. small-sized, non-colonial diatoms) in unregulated streams were replaced by colonial life forms, large-sized, and loosely attached diatoms in sites affected by dams in northern Spain. These contrasting results could be attributed to the summer flows, a key hydrological factor in the flow alteration dynamics that did not seem to affect diatom traits in our study. Other stressors, such as increases in nutrient levels and stronger flow change rates, caused significant community shifts in our study, favouring adnate life forms and reducing pedunculate ones. Pedunculate diatoms are able to exploit dissolved nutrients in the water and thus colonise nutrient-poor habitats with low flow disturbance (Pringle, 1990; Rimet and Bouchez, 2011); in contrast, adnate life forms adhered to the biofilm are highly resistant to repeated scour disturbances (Tornés et al., 2015).

In general, our findings might suggest that diatom traits might be less sensitive to seasonal flow inversions but respond to factors such as nutrient availability (Lange et al., 2011) and other types of flow disturbances (Wu et al., 2019b), e.g. flow change rates. However, we highlight that diatom responses to flow regime components may be confounded by the interactions between dam-related effects and environmental variability (Krajenbrink et al., 2019; Wagenhoff et al., 2017), e.g. differences in the altitude, land use and land cover, and geology among mountain ranges. Therefore, reducing the environmental variability would contribute to better assessing dam-related impacts.

In contrast to diatoms, macroinvertebrates did not show a global significant relationship between species distribution and the hydrological and physico-chemical variables. It highlights the potential use of diatom traits to assess the dam-related impacts over relatively broad spatial scales. Nevertheless, the individual significant trait-hydrology relationships identified in the macroinvertebrate analyses might be useful to understand the mechanistic effects of dam-related impacts. Traits better

adapted to the dynamic flow conditions (e.g. small body sizes, multiple reproductive cycles per year) in unregulated mountain streams were replaced by univoltine taxa, larger body sizes, larval aquatic stages, and active dispersion, as also reported in previous studies (Bonada and Dolédec, 2018; Piano et al., 2020). Moreover, hydrological changes related to the mountain range factor, such as the differences in the flow pulse patterns observed between the Pyrenees and the Iberian System, caused significant community changes in macroinvertebrate communities. It might indicate that spatial-related processes (e.g. dispersal and colonisation) and biogeography might have a stronger influence on macroinvertebrates (De Bie et al., 2012; Finlay, 2002), overriding the effects of the local hydrological and environmental conditions (i.e. niche).

4.4.4 Study limitations

This study relies on data originally collected for the water bodies' health assessment within the European WFD, as also seen elsewhere (e.g. Feeley et al., 2020; Kelly-Quinn et al., 2020; Krajenbrink et al., 2019). Posthoc analyses of these existing datasets may support hydroecological research; however, as observed in our study, several inherent challenges arise when using data surveyed for other purposes (Vaughan and Ormerod, 2010). The data heterogeneity and quality (i.e. surveys and taxonomic identification conducted by different water agencies in Spain), the temporal (annual survey campaigns) and spatial coverage (e.g. relatively few sampling sites paired to flow gauges), and the lack of relevant variables measured in the field (e.g. hydromorphological conditions, sediments or longer-term water quality) may often contribute to the uncertainties associated with biota responses to flow regulation.

In addition, as required by the lotic bioassessment protocols in Spain (e.g. IBMWP index; Alba-Tercedor et al., 2002; MAGRAMA, 2013), macroinvertebrate communities were mainly identified at the family level. Although family-level identification is reported to be cost-effective and suitable for bioassessment (Gayraud et al., 2003; Heino et al., 2007), the use of finer taxonomic resolution may be necessary to detect subtle impacts in macroinvertebrate communities (Chessman et al., 2007). Likewise, taxonomic resolution may cause significant differences in LIFE scores (Monk et al., 2012) and in the detection of the key environmental variables controlling community patterns (Jiang et al., 2013).

We also point out that the RLQ permutation tests failed to indicate a significant overall trait-environment association in macroinvertebrate communities, indicating that macroinvertebrate taxa distribution was independent of the environment or

functional traits. Even though all the selected sites were located in mountain areas, the environmental and hydrological heterogeneity among regions (e.g. variations in altitude, climate, geology, and hydrological characteristics) may produce substantial trait-based differences among communities at the regional scale (Bonada et al., 2007) and increase the noise in the analyses. Previous research has also reported that controlled settings produced more significant outcomes since the effect of confounding variables is reduced, allowing a direct investigation of the trait-environment response (Brown et al., 2019). Thus, future research should focus on the reduction of hydrological and environmental variability. Methods such as control-impact study design, grouping dams with similar characteristics (McManamay et al., 2016), and hydrological classification (Arthington et al., 2006) would reduce the noise arising from the inherent environment variability and, therefore, enable the analysis of dam-related impacts.

4.4.5 Implications for biomonitoring and conservation efforts

In contrast to taxonomic biotic indices, trait-based analyses allowed to establish mechanistic linkages of biotic responses to hydrological and physico-chemical conditions (Culp et al., 2011), such as changes in the diatom life forms and biovolume along the dam impact gradient, as also reported elsewhere (Murphy et al., 2017; Troia and McManamay, 2019). In combination with HIs, traits can provide a template to create and test large-scale hypotheses regarding flow-ecology responses (McManamay and Frimpong, 2015; Poff and Allan, 1995). Although research in this field is growing (e.g. Goldenberg-Vilar et al., 2021; Larsen et al., 2021; Nukazawa et al., 2020; Wu et al., 2019b), linkages between the flow regime and biological communities are usually not fully explored due to the lack of long-term discharge data and paired biological samples. Therefore, setting biomonitoring networks in which communities are sampled more frequently (e.g. covering seasonal variability), over a longer time frame, and with adequate spatial coverage (e.g. with paired river gauging) would be beneficial for monitoring and tackling dam impacts on river ecosystems, as well as for identifying future research priorities (Hausner et al., 2003).

Furthermore, exploring the responses of different organisms to dam-related impacts is essential to select meaningful biomonitoring metrics (Poff and Zimmerman, 2010). Diatom communities in our study showed, in general, to significantly respond to dam-related changes, whereas in macroinvertebrate communities these responses were not clear. The differences between diatoms and macroinvertebrates in terms of body size, life-history strategies, and dispersal capacity may have contributed to their responses to local environmental characteristics and stressors (De Bie

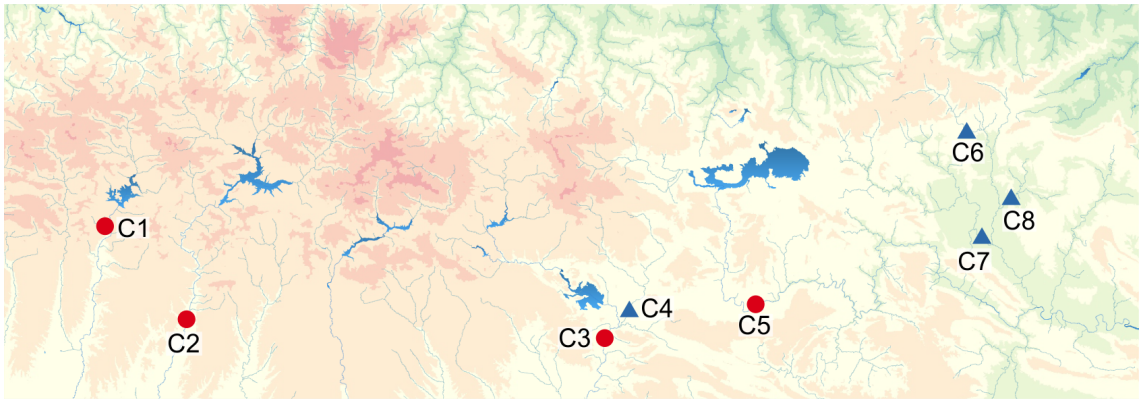
et al., 2012; Grenouillet et al., 2008; Shurin et al., 2009). Diatoms have good dispersal abilities and fast population growth rates and, therefore, are considered good bioindicators to track environmental change over large spatial scales (Bennett et al., 2010). Macroinvertebrates, in contrast, are weaker dispersers in comparison to diatoms, thus spatial-related processes may have a greater influence on their community structure than environmental control (Heino, 2013; Padial et al., 2014).

In conclusion, our findings highlighted the main hydrological alterations caused by irrigation dams and their effects on riverine biota, which varied among biotic groups, mountain ranges, and dams' hydrological alteration patterns. Trait-based analyses allowed to capture more complex changes in the communities (e.g. life form, body sizes, life history strategies) along the dam impact gradient than the taxonomic approaches. Diatoms showed to better respond to the presence of dams than macroinvertebrates, which could be linked to their lower spatial dependency. These results contribute to the development of efficient biomonitoring strategies (e.g. selection of suitable biotic groups and adequate spatial scales) and flow-ecology relationships (e.g. ecological responses to key HIs) as part of riverine conservation strategies (e.g. ELOHA environmental flow framework; Poff et al., 2010).

4.5 Supplementary Material

Table S4.1 Selected sampling sites divided by mountain range: Cantabrian Cordillera (C). Iberian System (I), and the Pyrenees (P), year of biological sampling, reservoir information (in the case of flow-altered sites), and the spatial distribution of sites in each mountain range. Reservoir purpose: Irrigation (IR), Hydropower (HP), Water Supply (WS).

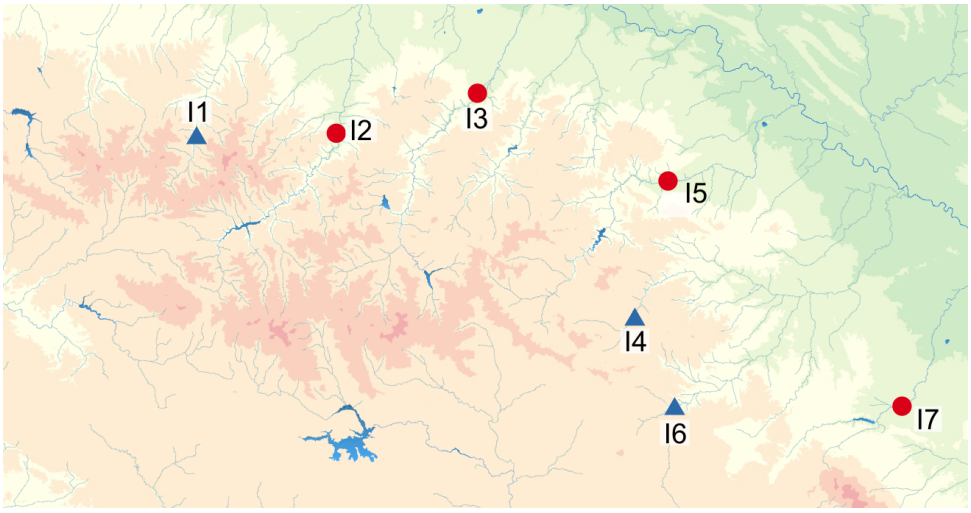
Site code	Year of biological sampling		Dam (reservoir volume)	Reservoir purpose	Distance dam-site
	Diatoms	Macroinv.			
C1	2017	2015	Juan Benet (318 hm^3)	IR/WS/HP	4.8 km
C2	2015	-	Riaño (664 hm^3)	IR/HP	30.4 km
C3	-	2016	Aguilar de Campoo (247 hm^3)	IR/HP	10.3 km
C4	-	2016			
C5	2015, 2016, 2017	2016, 2017	Ebro (540 hm^3)	IR/WS	35.2 km
C6	2015, 2017	2015, 2017			
C7	2015, 2016, 2017	2015, 2016, 2017			
C8	2015, 2017	-			



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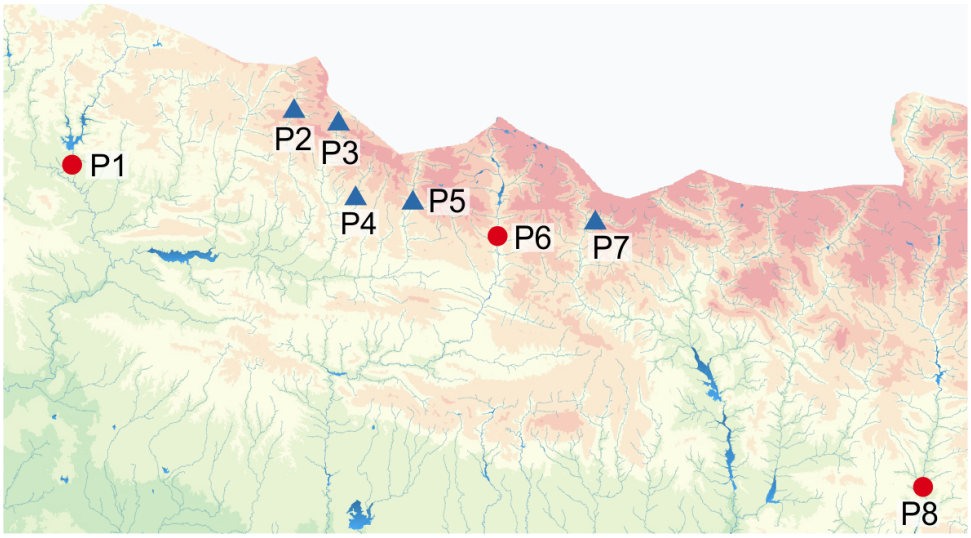
Site code	Year of biological sampling		Dam (reservoir volume)	Reservoir purpose	Distance dam-site
	Diatoms	Macroinv.			
I1	2015, 2017	2015, 2017			
I2	2016, 2017	2016, 2017	Mansilla (50 hm^3)	IR/HP	28.3 km
I3	2015, 2016, 2017	2015, 2016, 2017	González-Lacasa (33 hm^3), Pajares (35 hm^3)	IR, IR/HP	34.7 km
I4	2015, 2017	2015, 2017			
I5	2015, 2017	2015, 2016	Enciso (46 hm^3)	IR/WS/HP	23.7 km
I6	2015, 2017	2015, 2017			
I7	2015, 2016, 2017	2016, 2017	Val (23.7 hm^3)	IR	3.8 km



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Site code	Year of biological sampling		Dam (reservoir volume)	Reservoir purpose	Distance dam-site
	Diatoms	Macroinv.			
P1	2015, 2016, 2017	2015, 2016, 2017	Itoiz (418 hm^3)	IR/WS	2.7 km
P2	2015, 2017	2015, 2017			
P3	2015, 2017	2015, 2017			
P4	2015, 2017	2015, 2017			
P5	2015, 2016, 2017	2015, 2016, 2017			
P6	2015, 2016, 2017	2015, 2016	Lanuza (25 hm^3), Bubal (62 hm^3)	IR/HP, IR/HP	8.9 km
P7	2015, 2016, 2017	2015, 2016, 2017			
P8	2016, 2017	2016, 2017	Escales (120 hm^3)	IR/HP/WS	23.6 km



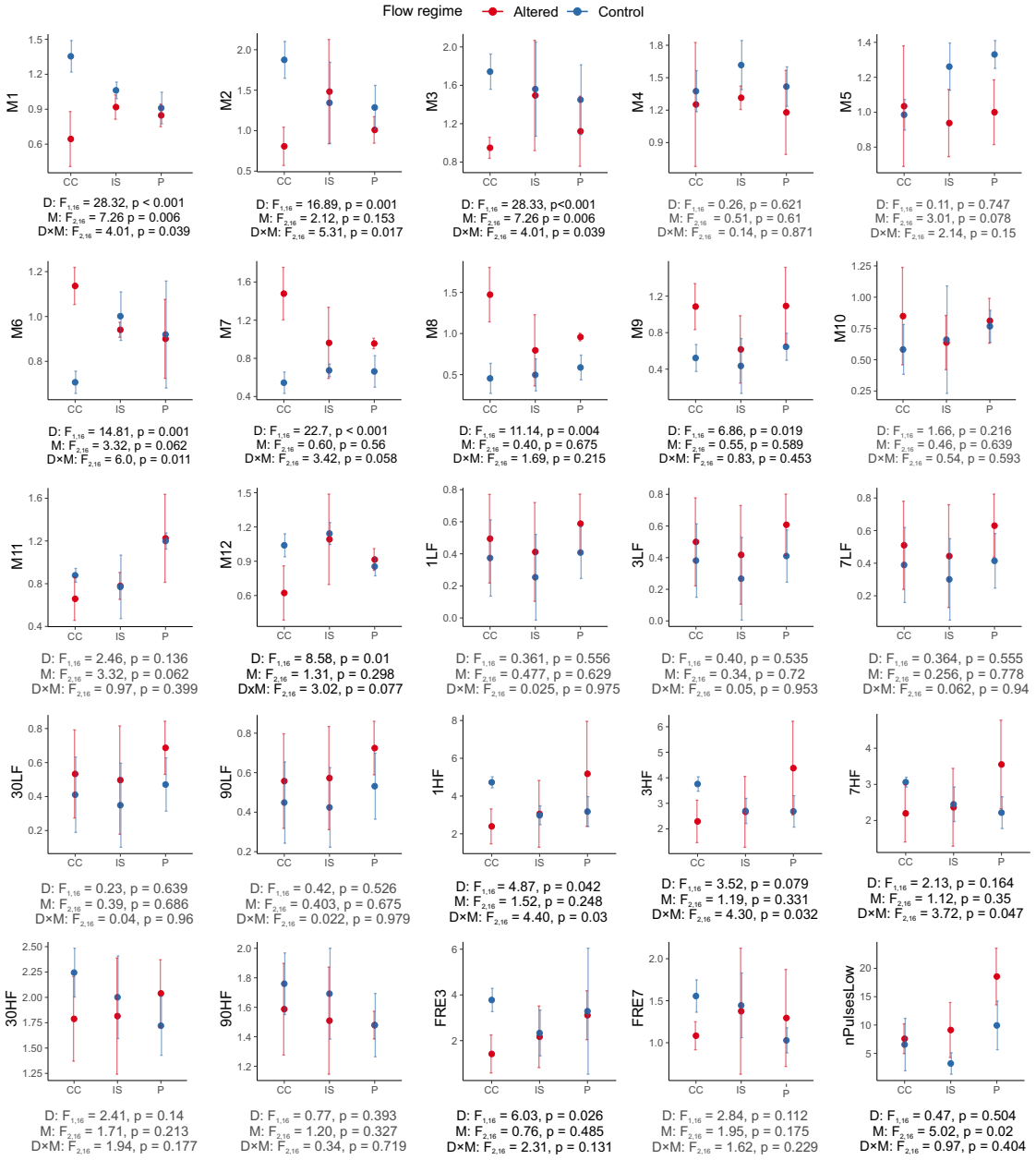


Figure S4.1 Interaction plots of hydrological indices and the two-way ANOVA tests in the Cantabrian Cordillera (CC), Iberian System (IS), and Pyrenees (P). "D" refers to ANOVA test with flow regime (dam presence) as a factor, "M" to mountain range as a factor, and "D×M" as the interaction between these factors. Vertical bars represent the standard deviation.

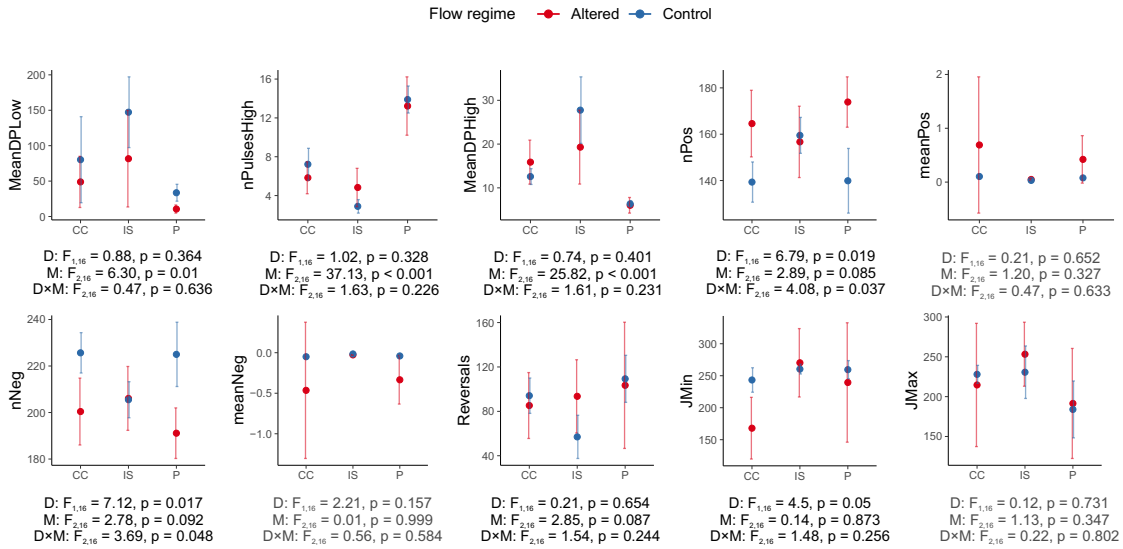


Figure S4.1 (cont.)

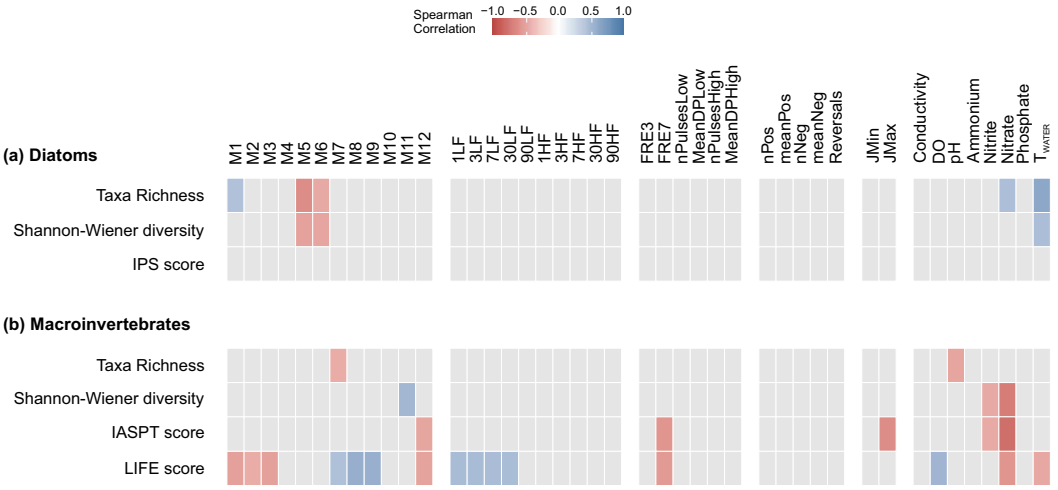


Figure S4.2 Correlation between biotic indices, hydrological, physico-chemical, and environmental variables using Spearman's rank test. Grey boxes indicate non-significant associations (p>0.05).

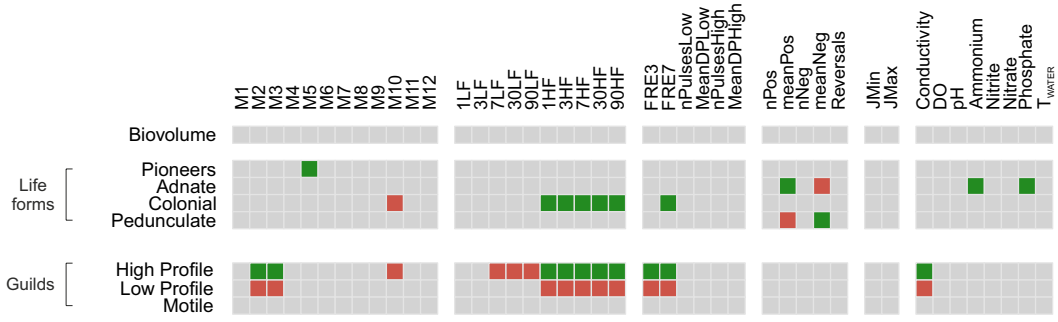


Figure S4.3 Fourth-corner correlations between diatom traits (rows), hydrological, and local physico-chemical variables (columns; see Table A.1 for codes). Grey boxes indicate non-significant correlations (p>0.05), red boxes negative significant correlations, green boxes significant positive correlations.

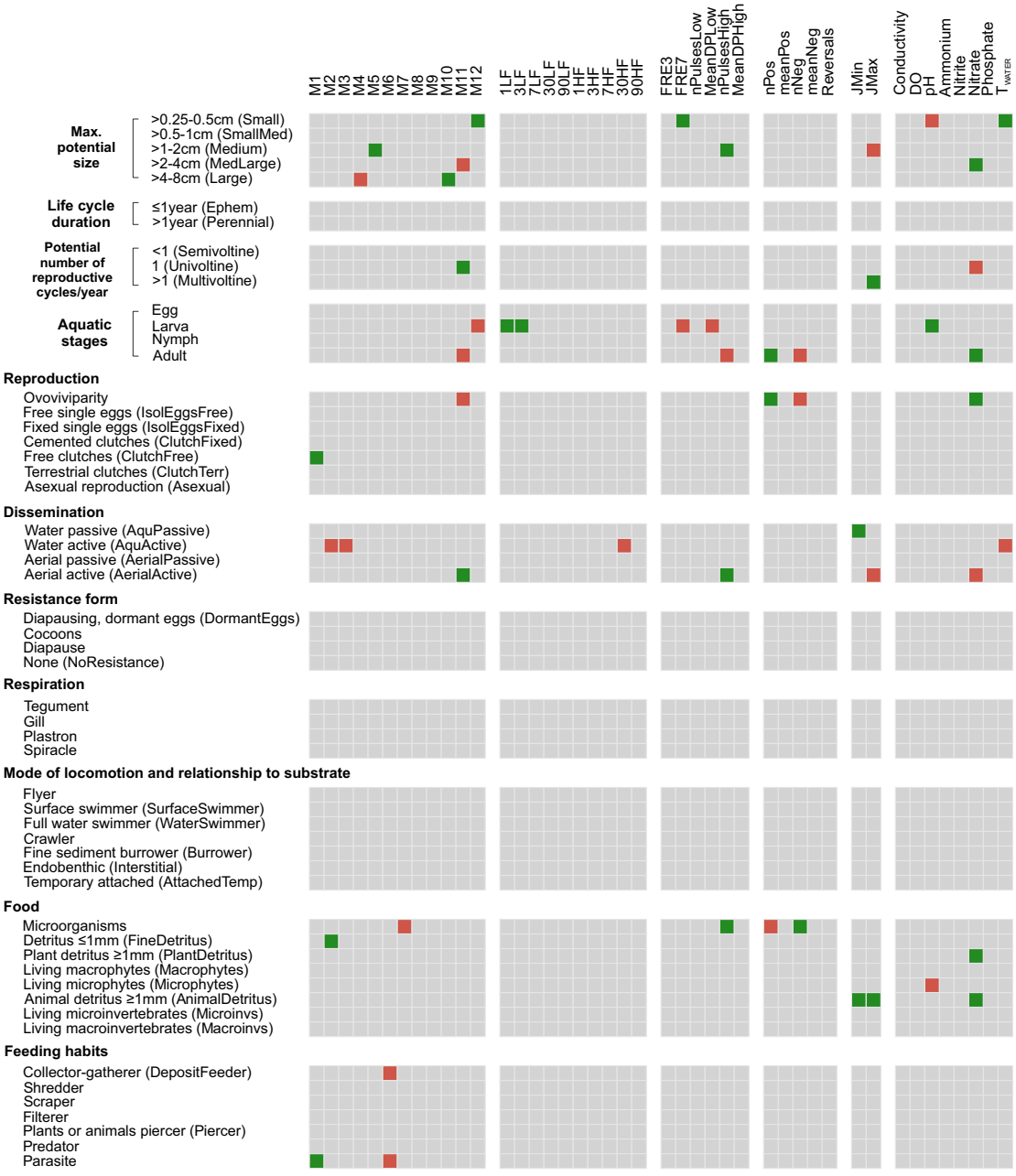


Figure S4.4 Fourth-corner correlations between macroinvertebrate traits (rows), hydrological, and local physico-chemical variables (columns; see Table A.1 for codes). Grey boxes indicate non-significant correlations (p>0.05); red boxes indicate negative significant correlations; green boxes indicate significant positive correlations.

Table S4.2 Diatom taxa list divided between controls and sites affected by irrigation dams (IR) in each mountain range. Values represent the mean ± standard deviation of abundances across samples.

Code	Species	Cantabrian Cordillera		Iberian System		Pyrenees	
		Control (n=4)	IR (n=3)	Control (n=5)	IR (n=7)	Control (n=7)	IR (n=5)
ADPY	*Achnanthidium pyrenaicum*	43.8 ± 18.4	140.9 ± 31.3	114.5 ± 99.4	44.3 ± 59.8	163.4 ± 126.2	87.8 ± 73
ADMI	*Achnanthidium minutissimum*	70.7 ± 35.9	78.1 ± 55.4	44 ± 20.3	40.4 ± 38.9	24.7 ± 17.9	20.1 ± 3.9
ACLI	*Achnanthidium lineare*	24 ± 41.6	2.3 ± 4	0.8 ± 1.4		48.7 ± 12	117.7 ± 103.9
CEUG	*Cocconeis euglypta*	20.3 ± 22.2	10.2 ± 4	6.3 ± 11	74.1 ± 51.9	4.5 ± 10.1	7.8 ± 10.2
COPL	*Cocconeis pseudolineata*			65.3 ± 113.2	42 ± 63.7		
GAGV	*Gomphonema angustivalva*			2.2 ± 3.8		34.5 ± 54.7	
GPUM	*Gomphonema pumilum*		0.7 ± 1.2	29.5 ± 51.1	13.2 ± 26.5	9.7 ± 18.5	4.2 ± 7.2
ENCM	*Encyonopsis microcephala*	2.5 ± 4.3		4.3 ± 4.5		2 ± 4.5	2.9 ± 5
CNTH	*Cocconeis neothumensis*	1.5 ± 2.6			20.2 ± 15		
NCTE	*Navicula cryptotenella*	11.2 ± 17.2	3.3 ± 5.8	0.3 ± 0.6	5.4 ± 3.4		2.8 ± 2.5
CAEX	*Cymbella excisa*	3.3 ± 5.8		3.3 ± 5.8	4.9 ± 9.9	11.6 ± 8.4	3.6 ± 3.8
ADSU	*Achnanthidium subatomus*			31.8 ± 55.1	±		
CPLI	*Cocconeis placentula var. lineata*		47.3 ± 80.3	0.3 ± 0.6	1.8 ± 3.5		
FSAP	*Fistulifera saprophila*		4.7 ± 5		2.4 ± 4.8		
GMIN	*Gomphonema minutum*	1.8 ± 3.1	5.6 ± 8		7.4 ± 14.8		
RSIN	*Reimeria sinuata*	8.5 ± 10.2		1 ± 1.7	0.4 ± 0.8	3.5 ± 5.6	0.5 ± 0.9
APED	*Amphora pediculus*	12.1 ± 19.3	5.4 ± 1.7	5.2 ± 4.5	4 ± 8		

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Table S4.2 – *Continued from previous page*

Code	Species	Cantabrian Cordillera		Iberian System		Pyrenees	
		Control (n=4)	IR (n=3)	Control (n=5)	IR (n=7)	Control (n=7)	IR (n=5)
NTPT	*Navicula tripunctata*	7.9 ± 13.7	2.1 ± 3.7		4 ± 2.8		
CPED	*Cocconeis pediculus*	4.5 ± 4.1	0.3 ± 0.6		1.8 ± 2.4		0.4 ± 0.8
EOMI	*Eolimna minima*	14.8 ± 25.6	2 ± 2		1.6 ± 3.2		
ACAF	*Achnanthidium affine*					7.7 ± 8.7	2.2 ± 3.8
ESUM	*Encyonopsis subminuta*					3 ± 5.4	3 ± 5.2
FPEM	*Fragilaria perminuta*					3.3 ± 5.2	±
DTEN	*Denticula tenuis*	8.9 ± 8.2		0.8 ± 1.4		0.7 ± 1.6	8.7 ± 10.3
ADEU	*Achnanthidium eutrophilum*	5.2 ± 9	7.4 ± 12.9				5.7 ± 9.8
ENMI	*Encyonema minutum*		7.7 ± 4.5		3.5 ± 5.2		
ESLE	*Encyonema silesiacum*	2 ± 1.9	4.7 ± 4.2		1.2 ± 2.5	0.4 ± 0.9	1.3 ± 2.3
NFON	*Nitzschia fonticola*	4.6 ± 7.9			1.8 ± 3.5		
NRCH	*Navicula reichardtiana*	2.6 ± 4.4	2 ± 3.5		5 ± 6.6		
NDIS	*Nitzschia dissipata*	4.3 ± 7.5	7.2 ± 12.5				
MAPE	*Mayamaea atomus var. permitis*	1.9 ± 3.3			2.2 ± 4.5		
UULN	*Ulnaria ulna*	6.7 ± 8.3			0.6 ± 1.2	0.2 ± 0.4	
GPAR	*Gomphonema parvulum*	1 ± 1.7					
BNEO	*Brachysira neoexilis*					3.2 ± 7.2	
NANT	*Navicula antonii*	7 ± 12.1			1.3 ± 2.7		
ALBL	*Adlafia lange-bertalotii*			0.8 ± 1.4			
ADGL	*Achnanthidium gracillimum*					5.1 ± 11.4	

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Table S4.2 – *Continued from previous page*

Code	Species	Cantabrian Cordillera		Iberian System		Pyrenees	
		Control (n=4)	**IR (n=3)**	**Control (n=5)**	**IR (n=7)**	**Control (n=7)**	**IR (n=5)**
AMID	*Amphora indistincta*	2.4 ± 4.2	1.2 ± 2.1				
PTLA	*Planothidium lanceolatum*			3 ± 5.2			
RABB	*Rhoicosphenia abbreviata*	1.3 ± 2.3	1.6 ± 2.7	0.7 ± 1.2			
ADRU	*Achnanthidium druartii*	1.6 ± 2.7	2 ± 3.5				
ADSB	*Achnanthidium straubianum*	1.8 ± 3.1					
DDEL	*Delicata delicatula*					2.6 ± 3.7	1.7 ± 2.9
NCPR	*Navicula capitatoradiata*	1 ± 1.7			1.2 ± 1.9		
FAUT	*Fragilaria austriaca*		3 ± 5.2			1.2 ± 1.2	1.6 ± 2.7
ADCT	*Achnanthidium catenatum*		2.3 ± 4				
GRHB	*Gomphonema rhombicum*			5 ± 8.7			
RUNI	*Reimeria uniseriata*	0.3 ± 0.6	0.4 ± 0.8		0.5 ± 1		
ESBM	*Eolimna subminuscula*		4.3 ± 7.5				
SIDE	*Simonsenia delognei*		0.9 ± 1.5				
DVUL	*Diatoma vulgaris*		12.3 ± 21.4				
GTER	*Gomphonema tergestinum*					0.7 ± 1.6	0.8 ± 1.4

Table S4.3 Macroinvertebrate taxa list divided between controls and sites affected by irrigation dams (IR) in each mountain range. Values represent the mean ± standard deviation of abundances across samples.

Families	Cantabrian Cordillera		Iberian System		Pyrenees	
	Control (n=3)	IR (n=3)	Control (n=3)	IR (n=4)	Control (n=5)	IR (n=3)
Baetidae	312.5 ± 467.5	1551.5 ± 2129.5	3206.3 ± 91.5	3593.3 ± 3738.1	1393.7 ± 955.3	1542.4 ± 399.9
Chironomidae	2101.6 ± 2906.1	1027 ± 1091.2	1909.3 ± 602	1082.6 ± 152.5	2141.5 ± 775.2	830.1 ± 400.5
Gammaridae	102.9 ± 81.8	462.5 ± 584.2	3045.3 ± 5270.3	1745.8 ± 1540		1272.6 ± 2197.3
Hydrobiidae	30.8 ± 36.6	0.2 ± 0.3	1362.3 ± 2359.6	2623.2 ± 2650.3	1.1 ± 2.1	
Simuliidae	98.9 ± 168.7	2257.8 ± 3801.7	676.5 ± 595.7	1112.2 ± 1242.8	444.2 ± 357.9	369 ± 327.7
Heptageniidae	123.4 ± 213.8	60.3 ± 97.6	375.7 ± 556.5	300.6 ± 253.2	1874.6 ± 1733.7	651.7 ± 865
Elmidae	90.9 ± 113	173.2 ± 262	352.3 ± 230.3	972.8 ± 777	531.6 ± 504.5	545.3 ± 712.1
Leuctridae	207 ± 298.5	224.7 ± 321.4	566.3 ± 596	177.6 ± 223.3	1421.4 ± 853.9	449.7 ± 555.1
Caenidae	702.5 ± 1211.6	8.5 ± 14.7	19.2 ± 16.9	661.9 ± 1315.8	119.9 ± 95.7	117.1 ± 184.3
Hydropsychidae	169.3 ± 258.8	183.8 ± 286.2	41.8 ± 45.9	230.4 ± 181.1	316 ± 386.1	291.7 ± 385.7
Oligochaeta	68.4 ± 54	130.7 ± 61.5	161.2 ± 214.7	212.7 ± 199.8	75.4 ± 42.6	52.9 ± 57.8
Ancylidae	10 ± 11.1	54.5 ± 50.9	70.7 ± 84.5	31.1 ± 19.9	4.1 ± 5.4	3.4 ± 6
Corixidae	2 ± 3.5	0.2 ± 0.3	17 ± 17.5	100.6 ± 179.4	265.5 ± 305.4	15.2 ± 19.8
Goeridae		0.2 ± 0.3	932.8 ± 1615.7	23.5 ± 46.7		0.1 ± 0.2
Ephemerellidae	3.1 ± 5.3	1132.2 ± 1808	309.5 ± 286.9	37 ± 33.6	94.5 ± 152.2	71.7 ± 53.8
Ostracoda		4.3 ± 7.5	65.3 ± 52.5	29.1 ± 16.2	7.7 ± 17.2	99.8 ± 138.3
Lymnaeidae		2.7 ± 4.6	18.7 ± 31.5	13.4 ± 21.2	90.1 ± 157.1	248.2 ± 214.5

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Table S4.3 – *Continued from previous page*

Families	Cantabrian Cordillera		Iberian System		Pyrenees	
	Control (n=3)	**IR (n=3)**	**Control (n=3)**	**IR (n=4)**	**Control (n=5)**	**IR (n=3)**
Perlidae		49.7 ± 86	41 ± 68.4	6.1 ± 12.2	168.7 ± 170.3	91 ± 138.2
Limnephilidae	0.8 ± 0.7	345.5 ± 575.6	23.5 ± 22.1	14.6 ± 15.8	123.9 ± 206.4	49.4 ± 44.9
Nemouridae	2.2 ± 3.8	0.3 ± 0.6	89.5 ± 155	2 ± 4	120.6 ± 138	6.3 ± 11
Leptophlebiidae	86.2 ± 117.4	2.7 ± 4.6	166.2 ± 226	1.3 ± 2.2	64.4 ± 64.2	4 ± 6.9
Hydroptilidae	36.1 ± 36.4	14.2 ± 23.7	8 ± 13.9	58.9 ± 61.3	25.9 ± 33.1	14.6 ± 12.9
Sphaeriidae		20 ± 34.6	104.5 ± 176.7	93.6 ± 81.7	1 ± 2.1	
Sericostomatidae		137 ± 237.3	48.5 ± 83.6	17.4 ± 24.4	88.4 ± 129	44.2 ± 65.9
Empididae	22.5 ± 33.9	15 ± 21.8	62 ± 36.5	20.2 ± 15	39.8 ± 42	4.2 ± 6.7
Polycentropodidae	65.6 ± 60.9	26.8 ± 35.3	29.8 ± 34.1	61.8 ± 90.7	5.8 ± 8.1	14 ± 24.3
Leptoceridae	48.2 ± 53.6		7.5 ± 8.4	16 ± 15	94.4 ± 197.9	0.1 ± 0.2
Dytiscidae	9.2 ± 15.2	4.3 ± 7.5	142.5 ± 118.1	17.7 ± 22.3	18.3 ± 12.4	3.2 ± 5.1
Hydraenidae	22.2 ± 38.4	0.2 ± 0.3	9.5 ± 9.6	4.7 ± 8.2	46.3 ± 41.7	0.3 ± 0.3
Rhyacophilidae	6.2 ± 10.8	24.7 ± 25.1	21.7 ± 30.7	17.9 ± 19.2	20.6 ± 15.2	53.8 ± 36.8
Athericidae		0.3 ± 0.6	1 ± 1.7	2 ± 3.7	94.9 ± 132.4	0.6 ± 0.7
Lepidostomatidae			6.7 ± 11.5	57.3 ± 105.5	3.6 ± 7.7	39.8 ± 68.9
Cambaridae		155.3 ± 269				
Ceratopogonidae		0.8 ± 1.4	7.7 ± 12.4	16 ± 32	14.4 ± 12	37.3 ± 64.7
Physidae				26.7 ± 53.1		
Asellidae				78.8 ± 157.6		
Psychomyiidae	18.4 ± 31.8	1.7 ± 2.9		15.7 ± 31.1	1.2 ± 2.7	8 ± 13.9

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Table S4.3 – *Continued from previous page*

Families	**Cantabrian Cordillera**		**Iberian System**		**Pyrenees**	
	Control (n=3)	**IR (n=3)**	**Control (n=3)**	**IR (n=4)**	**Control (n=5)**	**IR (n=3)**
Tabanidae			1.3 ± 1.3	0.3 ± 0.3	43.1 ± 74.5	18.9 ± 31.9
Glossosomatidae					.1	27.9 ± 48.3
Planariidae			0.2 ± 0.3		11.1 ± 12.8	11.4 ± 19.8
Brachycentridae			80.2 ± 138.9	8.1 ± 15.9	1.2 ± 2.7	
Gyrinidae			28.2 ± 46.6	3.1 ± 4.4	13 ± 16.5	24 ± 41.6
Pediciidae			55.8 ± 90.2		7.1 ± 8.4	1.1 ± 1.9

Chapter 5

Assessing irrigation and hydropower dam effects on riverine communities using taxonomic and multiple trait-based approaches

This chapter is an edited version of the research article published in the journal Ecological Indicators (145) by Cássia Rocha Pompeu, Francisco J. Peñas, Alejandra Goldenberg-Vilar, Mario Álvarez-Cabria, and José Barquín in 2022 with the title "*Assessing the effects of irrigation and hydropower dams on river communities using taxonomic and multiple trait-based approaches*". DOI: 10.1016/j.ecolind.2022.109662.

Abstract

Rivers and streams have suffered multiple transformations to attend to the increasing water demands worldwide. Among these, dams and reservoirs cause some of the most severe ecological impacts on rivers, altering the river flow and thermal regimes, nutrient and sediment fluxes, and network connectivity. However, in the context of the different dam purposes and operational schemes, knowledge of the ecological impacts on the riverine biota is still limited. In this study, our main goal was to assess dam-related effects (e.g. hydrological, thermal, and water quality changes)

on river biological communities and identify key ecological responses associated to it. To achieve this, diatom, macroinvertebrate, and fish communities were surveyed in control (n=8) and impacted (n=11) streams (i.e. downstream of irrigation or hydropower dams) over three consecutive years. The study design aimed at minimising the environmental variability among control and impacts using previously established hydrological classes. This allowed focusing primarily on the effects of dam operation schemes. In addition to traditional biotic indices based on the composition and structure of these communities, we assessed community-level responses using trait-based analyses with RLQ and fourth-corner analyses. The ecological changes varied with dam purpose and, in general, favoured disturbance-tolerant traits. Common biotic indices did not consistently respond to dam uses; trait-based analyses, in contrast, provided a more detailed picture of the dam-related effects on the studied river communities, with macroinvertebrate traits showing the strongest correlations to dam-related hydrological and physico-chemical variables, followed by diatoms and fish. Changes in the biological communities downstream of irrigation dams were mostly related to the inversion of the seasonal flow regimes (e.g. increases in the summer flows and magnitude of low flow extremes; decreases in the winter flows and in the frequency and magnitude of extreme high flow events) and the reduction of nutrient concentration. These changes favoured planktonic diatoms, macroinvertebrates with short life cycles and small body sizes, and fish feeding on the water column. Hydropower dams elevated significantly the rate of flow change and water temperature, favouring low profile adnate diatoms, multivoltine and passively dispersed macroinvertebrates, and scrapers. The key relationships identified in our study are useful to underpin river biodiversity conservation strategies and to set future research directions aiming at reducing the negative effects of irrigation and hydropower dams.

5.1 Introduction

Rivers and streams have been suffering a dramatic transformation to attend to the increasing human freshwater demand worldwide. A significant part of this transformation is related to the construction of dams and other infrastructures for water diversion and storage, electricity generation, and risk management (Grill et al., 2019; Malmqvist and Rundle, 2002), with pervasive effects on riverine ecosystems (Richter et al., 2003; Vörösmarty et al., 2010). Therefore, one of the primary challenges for water resource managers is balancing these infrastructure's impacts on the verge of society development, while meeting ecological demands aggravated by global change (Arthington et al., 2010; Tickner et al., 2020b).

Large dams represent the most prevalent form of hydrological alteration of river ecosystems worldwide (Arthington et al., 2010; Poff and Zimmerman, 2010; Rolls et al., 2013). Flow regulation caused by dams has been associated to the homogenisation of biological communities (e.g. Clavero and Hermoso, 2011; Oliveira et al., 2018), life cycle disruptions (e.g. Mims and Olden, 2013), and invasions of non-native species (e.g. Mathers et al., 2020). However, dams alter not only hydrological patterns, but also thermal regimes (Olden and Naiman, 2010; Poff and Zimmerman, 2010; Rolls et al., 2013), nutrient and sediment fluxes (Maavara et al., 2020; Syvitski, 2003), water quality (Scott Winton et al., 2019), and river longitudinal connectivity (Fagan, 2002; Grill et al., 2019). Despite the mitigation measures implemented worldwide to reduce dam impacts, such as fish passages, and environmental flow regimes (Arthington et al., 2010; Poff and Matthews, 2013; Schilt, 2007), global freshwater biodiversity is still declining at alarming rates (Baker et al., 2020; Liermann et al., 2012). Therefore, understanding the ecological effects produced by dams in a systematic way is paramount for developing effective conservation strategies such as specific recommendations to restore key aspects of the flow regime (Poff et al., 1997).

Dams tend to diminish peak flows and increase minimum flows, i.e. homogenise the flow regime, reducing channel complexity and, consequently, river biodiversity (Moyle and Mount, 2007; Poff et al., 2007). However, dam operational rules and storage capacity can influence specific hydrological and water quality variables (McManamay, 2014; Poff and Hart, 2002) and the ecological impact they produce. Despite numerous studies investigating how dams affect riverine assemblages (e.g. Chessman et al., 2010; Poff and Zimmerman, 2010; Sánchez-Pérez et al., 2020; Wu et al., 2019b), the impacts associated to different dam operational schemes are seldom investigated simultaneously (but see Ko et al., 2020; Salmaso et al., 2018). Irrigation dams capture high flows and release the storage water depending on agricultural water demands, which can completely rearrange flow seasonality (Richter and Thomas, 2007) and therefore alter taxonomic and functional biological community structure (e.g. White et al., 2017b). In contrast, hydropower dams can operate with little or no storage capacity, employing the river's natural flow range (i.e. "run-of-river" operation) and generating minor flow regime alterations (Egré and Milewski, 2002; Richter and Thomas, 2007). However, downstream of these small hydropower plants, habitat degradation, water quality deterioration, and connectivity loss cause important ecological impacts (Kuriqi et al., 2021). Hydro-peaking dams are a different case, with frequent short-term flow fluctuations according to energy demands, which can have enormous short- and long-term impacts on river biota (e.g. increased macroinvertebrate drift; Schülting et al., 2019).

Biotic indices are widely employed in the biological monitoring of water quality (Bonada et al., 2006) and have been also used to investigate the ecological effects of flow regulation. In general, most biotic indices (e.g. IASPT and EPT richness based on macroinvertebrate communities) were found to be sensitive to hydrological changes downstream of dams (e.g. Laini et al., 2018; Meißner et al., 2018; Mellado-Díaz et al., 2019), although these relationships lack a mechanistic or process-based background that limits their application to water management. Complementary, the biological communities' responses to dam-related disturbances (e.g. hydrological and physico-chemical changes) can also be approached through a trait-based analysis (Culp et al., 2011). Species traits, i.e. measurable organism features describing, for example, body size, fecundity, and ecological preferences (McGill et al., 2006), have demonstrated consistent responses to environmental and hydrological variation across taxonomic boundaries (Frimpong and Angermeier, 2010; Göthe et al., 2017) and provide a better mechanistic understanding of the community responses to natural and anthropogenic stressors (Statzner and Bêche, 2010; White et al., 2017b). For instance, the dominance of different macroinvertebrate feeding groups (Larsen et al., 2021) or different fish life strategies (Oliveira et al., 2018) are reported to vary significantly in response to flow alteration. These patterns can be readily translated into flow-ecology relationships (Craven et al., 2010; Rosenfeld, 2017) and, in combination with river classification, underpin conservation strategies applicable beyond the regional species pool (Arthington et al., 2018b; Poff et al., 2010). These strategies (e.g. specific environmental flows) are critical to sustain fluvial ecosystems while addressing competing objectives such as water allocation for agriculture and energy production (Kuriqi et al., 2020; Poff and Matthews, 2013).

In this study, our main goal is to assess dam effects (e.g. hydrological and thermal alteration, water quality changes) on multiple river biological communities, focusing on dams primarily operated to attend to irrigation demands or hydropower production. We aim to identify consistent community changes and trait-based ecological responses using diatom, macroinvertebrate, and fish communities (i.e. organisms representing different levels of the river food web) in a control-impact design (natural vs. regulated flow regime) in northern Spain. The selected streams are grouped into two natural hydrological classes (following Peñas and Barquín, 2019), reducing environmental and hydrological variability. We hypothesise that (1) flow regulation will alter hydrological and physico-chemical characteristics depending on the dam purpose (i.e. irrigation vs. hydropower dams), and (2) both biotic indices and ecological traits will respond to these changes, however, differently in each biotic group and depending on the intensity of hydrological or physico-chemical change. In general, we expect to identify key community shifts associated to the hydrological

and physico-chemical changes, (3) with dams favouring disturbance-tolerant traits (e.g. generalist taxa, small body sizes, etc).

5.2 Methodology

5.2.1 Study design

Spain is among the top ten countries in dam building, with 1,064 large dams (with a minimum height of 15 m or impounding more than 3 hm^3), according to the International Commission on Large Dams (ICOLD, 2021). River damming in the country has been motivated by the high water agriculture demands (38,779 km^2 of irrigated lands; MAPAMA, 2021), low and irregular rainfalls, and high potential evapotranspiration (Fornés et al., 2021). Although hydropower expansion decelerated in the last decades due to environmental legislation and the exploitation of other energy sources (Fornés et al., 2021), 147 new hydropower plants are planned to be built in the country, mostly with small energy generation capacities (<10 MW; Schwarz, 2019).

In this study, we selected 19 permanent streams in northern Spain surveyed in three consecutive autumns (2017, 2018, 2019): 8 streams under natural flow regime (i.e. controls) and 11 streams affected by dams (i.e. altered flow regime; Figure 5.1a and Table 5.1). We focused on dams operating with two main purposes: dams primarily used for irrigation and dams operated solely for hydropower production. Sampling sites were located in the Cantabric, Duero, and Ebro catchments, which drain to the Cantabric Sea, the Atlantic Ocean, and the Mediterranean Sea, respectively. The initial condition for site selection was to have an operational nearby gauge station. In potentially impacted sites (i.e. streams under a regulated flow regime caused by a reservoir located upstream), we selected river reaches affected by a minimum of one and a maximum of three upstream reservoirs.

Our sampling sites were restricted to streams that belong to natural hydrological classes 1 (NC1) and 2 (NC2). These two groups correspond, respectively, to NC10 and NC13 in the 20-level hydrological classification developed by Peñas and Barquín (2019) for the Spanish river network. The natural flow regime of NC1 streams was characterised by a large number of high flow events with short duration and low interannual variability on the timing and magnitude of low flows (Figure 5.1b). NC2 was also characterised by a large number of high flow events, with a peak in spring associated to snowmelt processes (Figure 5.1c-d). Although the hydrological classes may not be a fixed product as their limits represent a trade-off between detail and

interpretability (Poff et al., 2010), they are able to reduce the noise associated to environmental and hydrological variability.

Table 5.1 Selected sampling sites divided between the hydrological classes NC1 and NC2. Reservoir purpose: Hydropower (HP), Irrigation (IR), Water Supply (WS). Storage index: storage volume/average annual runoff.

	River (Code)	Basin	Dam (year of construction)	Reservoir purpose	Storage Index
NC1	Omaña (OMA)	Duero			
	Cea (CEA)	Duero			
	Tirón (TIR)	Ebro			
	Ega (EGA)	Ebro			
	Ebro (EBR)	Ebro	Ebro (1945)	IR/WS	3.71
	Duero (DUE)	Duero	Cuerda del Pozo (1941)	IR/WS/HP	0.74
	Luna (LUN)	Duero	Barrios de Luna (1956)	IR/HP	0.84
	Arbejal (ARB)	Duero	Requejada (1935)	IR/HP	0.46
	Carrión (CAR)	Duero	Camporredondo (1930), Compuerto (1960)	IR/HP	0.72
NC2	Sella (SEL)	Cantabric			
	Deva (DEV)	Cantabric			
	Curueño (CUR)	Duero			
	Bernesga (BER)	Duero			
	Nansa (NAN)	Cantabric	La Cohilla (1950), Palombera (1955)	HP	0.06
	Nalón (NAL)	Cantabric	Tanes, Rioseco (1978)	HP	0.13
	Narcea (NAR)	Cantabric	La Barca (1966)	HP	0.03
	Porma (POR)	Duero	Juan Benet (1968)	IR/WS/HP	1.64
	Esla (ESL)	Duero	Riaño (1987)	IR/HP	0.86
	Arlanzón (ARL)	Duero	Arlanzón (1933), Uzquiza (1988)	IR/WS	0.63

5.2.2 Environmental and hydrological information

Environmental characteristics describing climate, topography, geology, and land use and land cover at the catchment scale (Table 5.2) were obtained from a synthetic river network (SRN), which was inferred from a 10-meter digital elevation model. The SRN is composed of virtual watersheds integrating the spatial river network with the environmental information at the national level (Barquín et al., 2015).

The river discharge was recorded in 19 gauge stations provided by the Spanish Ministry for Ecological Transition and Demographic Challenge (CEDEX, 2022). We

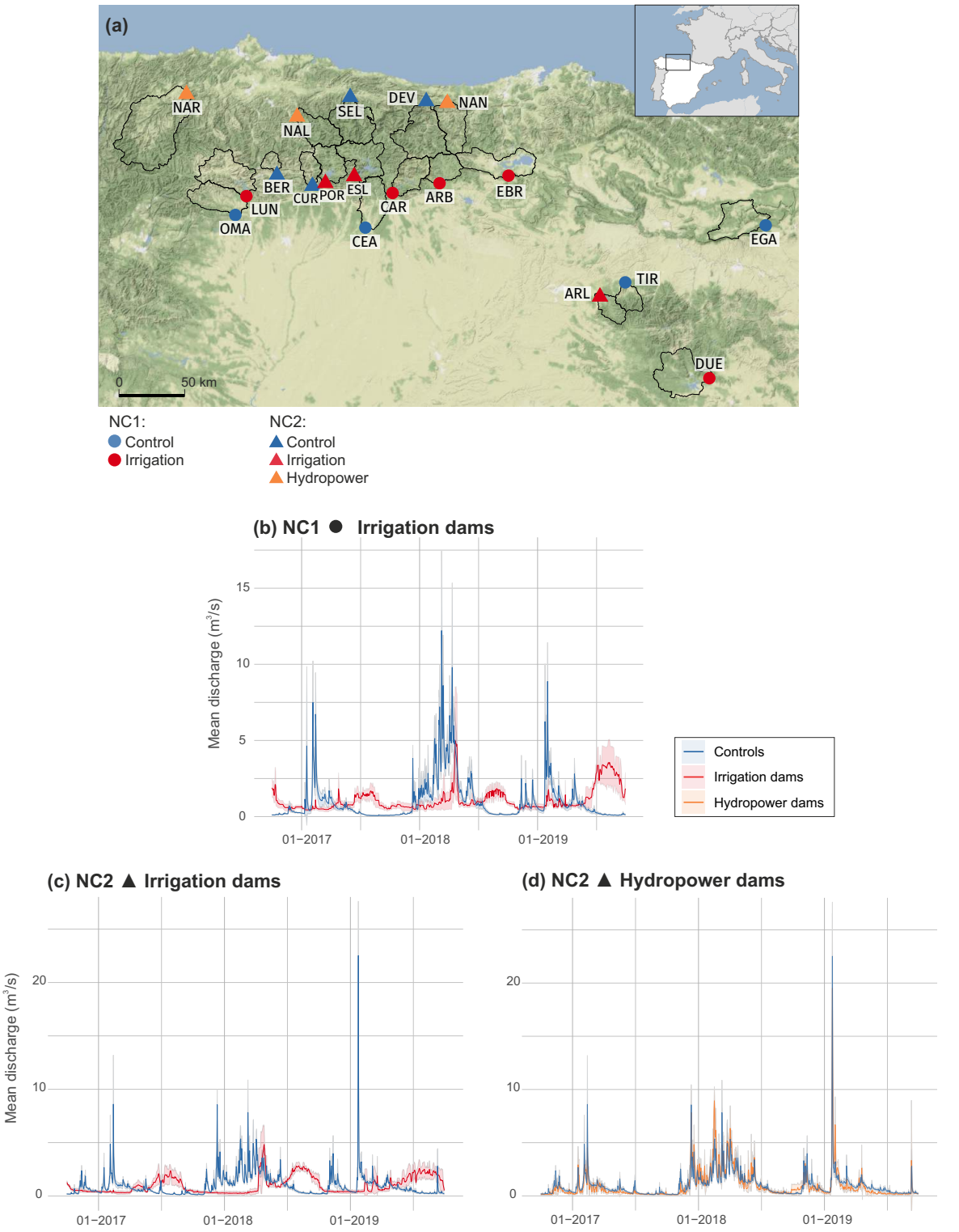


Figure 5.1 (a) The 19 sampling locations and their catchment boundaries, classified according to the flow regime: unregulated (control) and altered flow regimes, and hydrological classes (NC1 and NC2). See Table 5.1 for site codes and Table 5.2 for environmental characteristics. Mean normalised daily discharge in (b) controls and sites impacted by irrigation dams in NC1, (c) controls and sites impacted by irrigation dams in NC2, and (d) controls and sites impacted by hydropower dams in NC2. Shaded areas represent 1SD confidence intervals.

Table 5.2 Catchment-scale environmental variables describing topography, climate, and fractions occupied by land use and land cover and lithology in the sampling sites belonging to NC1 and NC2. Values refer to the catchment mean ± sd upstream of the sampling site.

	NC1		NC2	
	Control (4 sites)	**Altered (5 sites)**	**Control (4 sites)**	**Altered (6 sites)**
Catchment size (km^2)	354.1 ± 117.1	429.7 ± 138.6	344.8 ± 261.3	510.0 ± 393.6
Distance to river outlet (km)	622.4 ± 64.5	781.2 ± 125.6	307.2 ± 333.6	351.2 ± 338.9
Elevation above sea level (m)	790.4 ± 212.5	993.8 ± 120.6	563.5 ± 602.2	585 ± 463.9
Annual mean precipitation (mm)	1055.3 ± 9.4	971.8 ± 139.2	1177.5 ± 120.6	1246.7 ± 108.4
Annual mean temperature (°C)	10.1 ± 1.5	8.6 ± 0.9	8.2 ± 1.7	9.7 ± 2.2
Annual mean evapotranspiration (mm)	506.2 ± 55.7	505.76 ± 51.7	448.4 ± 14.5	491.7 ± 15.1
Urbanisation	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Agriculture	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0
Pastures	0.2 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
Broadleaf forests	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.3 ± 0.1
Coniferous forests	0 ± 0	0.1 ± 0.2	0 ± 0	0 ± 0
Plantations	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0.1
Heathlands and shrubs	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
Denuded areas	0 ± 0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Calcareous rocks	0.2 ± 0.3	0.3 ± 0.4	0.2 ± 0.2	0.2 ± 0.2
Conglomerate rocks	0.3 ± 0.2	0.3 ± 0.3	0.6 ± 0.2	0.4 ± 0.2
Sandstone	0 ± 0	0.2 ± 0.2	0 ± 0	0 ± 0
Sedimentary rocks	0.1 ± 0.2	0 ± 0	0 ± 0	0 ± 0
Siliceous rocks	0.2 ± 0.3	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.3
Slates	0.2 ± 0.3	0 ± 0	0 ± 0	0.1 ± 0.1
Volcanic rocks	0 ± 0	0 ± 0	0 ± 0	0 ± 0

extracted the average daily discharge corresponding to the hydrological years of 2017, 2018, and 2019 (Figure 5.1b-d). These years presented important meteorological differences, i.e. 2017 was considered a dry year, 2018 a wet year, and 2019 an average year according to the Spanish Governmental Agency of Meteorology (AEMET). Therefore, we computed 35 hydrological indices (HIs, Table A.1 in Appendix A) annually using the normalised daily flow and describing the magnitude of monthly flows, the magnitude, duration, and timing of annual extremes, the frequency and duration of high and low pulses, and the rate of change (Richter et al., 1996).

5.2.3 Biological and physico-chemical data collection

Diatom, macroinvertebrate, and fish biological communities were surveyed annually between September and October 2017, 2018 and 2019, when all sites presented low flow conditions. This is crucial because low flow comparison is unfeasible at other periods of the year since streams affected by irrigation dams usually have high flows in summer, whereas control sites typically have high flows in winter and spring.

Diatoms were surveyed by selecting randomly five to ten cobbles from the benthos (approximately 100 cm^2 of exposed surface area) and scrubbing the upper part of the substratum with a dishwasher brush (CEN, 2014a). The material was decanted in a sample bottle and preserved using formaldehyde. From each sample, 400 diatom valves were identified in laboratory using a microscope at the lowest feasible taxonomic level (species level in almost all cases).

Macroinvertebrates were surveyed from run and pool habitats using a Surber net (CEN, 2012). The stream substrate enclosed in the Surber sampling area (0.09 m^2) was disturbed and the material was swept into the net (0.5 mm mesh size) by the stream flow. Each sample (run and pool) was composed of three surbers. The taxonomic composition was completed with a kick hand-net sampling carried out in other habitats presented in the river stretch (e.g. roots, macrophytes, woody debris, etc.) with 10 kicks. Samples were preserved in 96% ethanol and identified in laboratory at the lowest feasible taxonomic level (mainly at species or genus level), except for Hydracarina, Nematoda, Ostracoda, Copepoda, and Oligochaeta.

Three days after the macroinvertebrate surveys, single-pass electrofishing (CEN, 2003) was carried out in a representative area of the river reach (670 m^2 on average) using a portable electric fishing device (power 1.3 kW, current generation of 300-500V or pulsations up to 940 V, and variable frequency 25-100 Hz). Nets or natural barriers were used to block upstream and downstream of the sampled area. After pass removal, the fish captured were kept in oxygenated boxes, anaesthetised with eugenol, counted, and identified at the species level. After a 30-minute recovery in containers with stream water, fish were returned alive to the stream. Brown trout (*Salmo trutta*) was classified into age classes: young of the year (0+), juvenile (1+) and adult (2+). Because of high flow conditions, Esla and Luna were not surveyed in 2018 and 2019, respectively.

To reduce the effect of rare taxa, we eliminated diatom and macroinvertebrate taxa representing less than 2% of total sampled individuals when considering the total number of occurrences (Lavoie et al., 2009). Regarding traits, we assigned diatom species to ecological guilds (high and low profile, planktonic, motile), life

forms (colonial, stalked, adnate, pioneer), and cell sizes (biovolume in μm^3; Table B.1 in Appendix B). Macroinvertebrates were assigned to traits describing their body size, life cycle duration, and feeding habits, among other characteristics (see Table B.2 in Appendix B), using a fuzzy-coding approach. Last, fish species were assigned to traits describing maximum body length, habitat use, feeding habits and habitat, and migration patterns (see Table B.3 in Appendix B). It is important to note that trait categories can overlap and species may belong to multiple trait categories (e.g. *Cocconeis euglypta* is considered, at the same time, a low profile and adnate diatom species).

Water physico-chemical measurements were collected during the field surveys. pH and conductivity were measured with Milwaukee pH55 and EC59 Portable Waterproof pH and EC/TDF Tester, respectively. The temperature was recorded with HOBO Pendant data loggers deployed in each site for at least 10 days between September and October each year. Nutrient concentrations were determined by continuous flow analysis (FIA and CFA) following standard protocols: UNE-EN ISO 15681-2:2005 for phosphates, UNE-EN ISO 13395:1997 for nitrite and nitrates, UNE-EN ISO 11732:2005 for ammonium, and UNE-EN ISO 77051:2019 for silicates.

5.2.4 Data analysis

An initial assessment of the relationships among the hydrological and physico-chemical variables in each hydrological class was performed using Spearman's rank correlations.

To identify significant differences in HIs, physico-chemical variables, and biotic indices between control and impacted sites in NC1 and NC2, we carried out a repeated-measure analysis of variance (ANOVA) using the *R* package `lme4` (Bates et al., 2015). Flow regulation was treated as a fixed factor, while the year of sampling as a random factor to account for potential temporal autocorrelation. In NC2, we further investigated the differences between controls, irrigation, and hydropower dams. The posthoc pairwise comparisons of the means were assessed by Tukey tests (`emmeans` *R* package; Lenth, 2021).

As the temperature is strongly influenced by altitude, the water temperature at sampling (T_{WATER}), the mean water temperature 14 days before biological sampling ($T_{14\text{-}DAY}$), and the degree-day accumulation in 10 days in October (Degree-day) were analysed in two groups in NC2: sites located at higher altitudes (>950m; Bernesga, Curueño, Porma, Esla, and Arlanzón), and sites at lower altitudes (<400m; Deva, Sella, Narcea, Nansa, and Nalón).

We calculated the taxa richness and Shannon-Wiener diversity index for the three biotic groups using the *R* package `vegan` (Oksanen et al., 2019). Additionally, we computed the Specific Polluosensitivity Index (IPS; CEMAGREF, 1982) for diatoms, the Iberian Average Score per Taxon (IASPT) and the Lotic invertebrate Index for Flow Evaluation (LIFE; Extence et al., 1999) for macroinvertebrates, and the European Fish Index (EFI+; EFI+ Consortium, 2009). Statistically significant relationships ($p \leq 0.05$) among these biotic indices, HIs, and physico-chemical variables were assessed using Spearman's rank correlations.

To investigate community variation across hydrological and environmental gradients, we carried out the RLQ and fourth-corner analyses (Dray et al., 2014; Kleyer et al., 2012) using the *R* package `ade4` (Dray and Dufour, 2007). The RLQ analysis combines three tables: table R ($n \times m$), containing the measurement of m environmental variables at n sites; table L ($n \times p$), with the abundance of p taxa at n sites; table Q ($p \times s$), describing s traits related to the same p taxa (Dolédec et al., 1996). This method is based on the computation of a cross-variance matrix weighted by taxa abundance, providing a broad overview of how traits and environmental characteristics are related (Brown et al., 2014; Thioulouse et al., 2018). The overall significance of the trait-environment relationship is assessed with 9999 permutations of the rows in tables R and Q sequentially to control type I error (ter Braak et al., 2012). Further, the fourth-corner analysis was used to identify significant individual trait-environment relationships using the three tables simultaneously. The method computes a matrix ($s \times m$) with the one-to-one trait-environment correlations (Dray and Dufour, 2007).

Prior to the analyses, water physico-chemical and hydrological variables were standardised (zero mean and unit standard deviation). The statistically significant level for all the ANOVA tests, Spearman's rank correlation, and RLQ and fourth corner analyses was set to $p \leq 0.05$. All the analyses were performed in the *R* environment (R Core Team, 2020).

5.3 Results

5.3.1 Dam effects on hydrology and water quality

In general, hydrological (Table 5.3 and Figure S5.1 in Supplementary Material) and water physico-chemical variables (Figure 5.2) responded differently to the presence of irrigation and hydropower dams, but similar patterns of alteration were identified in both hydrological classes.

In both natural hydrological classes, the presence of irrigation dams generated significant increases in summer flows (+M7-10, see Table 5.3) and in the magnitude of daily to seasonal low flow extremes (+1-90LF in NC1; +1-30HF in NC2). These HIs were correlated (Spearman's rank correlations in Figure S5.2 in Supplementary Material) to a higher number of days with increasing flows (+nPos), and lower nutrient concentrations (-Silicate, -Phosphate, -Nitrate in NC1; -Silicate in NC2).

We also identified a significant decrease in winter flows (-M1-3 in NC1; -M1-3, -M12, in NC2), lower frequencies of high flow events (-FRE3-7), lower magnitudes of short-term high flow extreme events (-1,3HF in NC1; -1,3,7HF in NC2), the earlier timing of annual 1-day flow extremes (-JMax in NC1), and a lower water conductivity and nutrient concentrations (-Nitrate, -Phosphate, -Silicate in NC1). These HIs were also correlated to lower flow rise (-meanPos) and less negative flow fall (+meanNeg) rates. Thus, the presence of irrigation dams resulted in an inversion of the seasonal flow patterns, decreases in the flow peak amplitude, and reductions in nutrient concentrations. These hydrological changes are clearly seen in the hydrographs (Figure 5.1b-c), e.g. in 2018, the high flow events magnitude was strongly reduced, and in 2019, summer flows were substantially higher in comparison to controls.

In streams affected by hydropower dams, the flow regime alteration was less evident (Figure 5.1d). A significantly higher number of low flow pulses within a year (+nPulsesLow; Table 5.3) but with shorter duration (-meanDPLow) were detected downstream of these dams. These HIs were also linked to lower spring flows (-M4-6), a lower number of days with decreasing flows (-nNeg), indicating a substantial day-to-day flow variation caused by these dams, as well as higher water temperature when compared to controls at similar altitudes (<400m; Figure 5.2).

Table 5.3 Standardised hydrological indices (mean ± standard deviation) in NC1 and NC2. See Table A.1 for HIs abbreviations. Statistically significant differences (Tukey pairwise comparisons) are represented as "⋘" $p \leq 0.001$; "≪" $p \leq 0.01$; "<" $p \leq 0.05$; "=" non-significant differences.

HI	NC1			NC2			
	C (n=12)	IR (n=15)		C (n=12)	IR (n=15)	HP (n=9)	
M1	1.3 ± 1.1	0.6 ± 0.3	IR=C	1.4 ± 0.8	0.4 ± 0.2	1.6 ± 0.9	IR⋘C=HP
M2	2.6 ± 0.9	0.8 ± 0.6	IR⋘C	2.1 ± 0.8	0.5 ± 0.4	2.4 ± 1.1	IR⋘C=HP
M3	2.4 ± 2.3	0.8 ± 0.9	IR≪C	1.7 ± 0.9	0.6 ± 0.5	1.7 ± 1.4	IR⋘C=HP
M4	2.3 ± 2.1	1.5 ± 1.9	IR=C	1.5 ± 1.1	1.4 ± 1.4	1.6 ± 1.4	IR=C=HP
M5	1 ± 0.5	0.7 ± 0.4	IR=C	1 ± 0.4	1 ± 0.4	0.8 ± 0.6	HP<C=IR
M6	0.8 ± 0.7	1 ± 0.5	C=IR	0.8 ± 0.5	1.4 ± 0.7	0.8 ± 0.7	HP=C<IR
M7	0.3 ± 0.3	1.5 ± 1.2	C≪IR	0.5 ± 0.2	2 ± 0.7	0.4 ± 0.2	HP=C⋘IR
M8	0.1 ± 0.1	1.7 ± 1.4	C⋘IR	0.2 ± 0.1	2.1 ± 0.8	0.2 ± 0.1	HP=C⋘IR
M9	0.1 ± 0.1	1.2 ± 0.9	C⋘IR	0.3 ± 0.2	1.3 ± 0.8	0.3 ± 0.2	HP=C⋘IR
M10	0.1 ± 0	1 ± 0.7	C⋘IR	0.2 ± 0.1	0.6 ± 0.2	0.3 ± 0.4	HP=C⋘IR
M11	0.5 ± 0.4	0.6 ± 0.4	C=IR	1.1 ± 0.6	0.4 ± 0.2	0.8 ± 0.4	IR⋘C, HP<C
M12	0.7 ± 0.4	0.6 ± 0.3	IR=C	1.1 ± 0.8	0.4 ± 0.2	1.2 ± 1.1	IR≪C=HP
1LF	0.1 ± 0	0.3 ± 0.2	C⋘IR	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	HP=C≪IR
3LF	0.1 ± 0	0.3 ± 0.2	C⋘IR	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	HP=C≪IR
7LF	0.1 ± 0	0.3 ± 0.2	C⋘IR	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	HP=C≪IR
30LF	0.1 ± 0	0.3 ± 0.2	C⋘IR	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	HP=C<IR
90LF	0.1 ± 0	0.3 ± 0.2	C⋘IR	0.2 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	HP=C<IR

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Table 5.3 – *Continued from previous page*

HI	NC1			NC2			
	C (n=12)	**IR (n=15)**		**C (n=12)**	**IR (n=15)**	**HP (n=9)**	
1HF	13.3 ± 5.9	5.1 ± 5.2	IR<C	14 ± 8.7	3.8 ± 1.8	11.7 ± 7.8	IR≪C=HP
3HF	9.8 ± 4.4	4.9 ± 5.1	IR<C	9.2 ± 5.3	3.6 ± 1.7	8.7 ± 4.4	IR≪C=HP
7HF	7 ± 3.2	4.4 ± 5	IR=C	5.7 ± 2.6	3.5 ± 1.8	6.1 ± 2.7	IR<C=HP
30HF	4.1 ± 2	2.9 ± 1.8	IR=C	3 ± 1	2.8 ± 1	3.3 ± 1.3	IR=C=HP
90HF	2.6 ± 1.5	2.4 ± 1.1	IR=C	2 ± 0.7	2.1 ± 0.5	2.1 ± 1.1	C=IR=HP
FRE3	4.5 ± 1.4	2 ± 1.1	IR⋘C	5.5 ± 1.6	2.5 ± 1.1	4.6 ± 1.6	IR⋘C=HP
FRE7	3.4 ± 1.7	1.6 ± 0.6	IR≪C	3.3 ± 1.6	2.3 ± 1.4	3.9 ± 1.5	IRC=HP
nPulsesLow	3.4 ± 1.7	4.9 ± 4.6	C=IR	5.5 ± 3.6	8.1 ± 8.7	14.1 ± 13.2	IR=CHP
meanDPLow	108.4 ± 76.5	99.3 ± 76.8	IR=C	66 ± 29.5	66.7 ± 41	35.2 ± 29.6	HP≪C=IR
nPulsesHigh	5.6 ± 2.5	6.7 ± 5.5	C=IR	11.2 ± 3.5	4.8 ± 1.7	11.3 ± 5	IR⋘C=HP
meanDPHigh	24.3 ± 26.8	24.5 ± 28.1	C=IR	8.3 ± 5.2	22.1 ± 10.4	7.9 ± 5.4	HP=C⋘IR
meanPos	1.7 ± 1.7	0.4 ± 0.5	IR=C	3.6 ± 3.6	0.6 ± 0.6	4.7 ± 3.7	IR<C=HP
nPos	126.5 ± 14.6	150.1 ± 32.9	C=IR	115.8 ± 13.6	173.6 ± 17.3	149.6 ± 31.1	C⋘IR, C≪HP
meanNeg	-0.8 ± 0.8	-0.3 ± 0.4	C=IR	-1.9 ± 2	-0.5 ± 0.4	-4.1 ± 3.4	HP=C=IR
nNeg	238.2 ± 14.5	196.8 ± 30.7	IR⋘C	248.7 ± 13.8	191.2 ± 17.1	197.9 ± 41.6	IR⋘C, HP⋘C
Reversals	101.3 ± 17.3	125.4 ± 25.1	C=IR	120.4 ± 15.6	148 ± 26.6	135.7 ± 31.8	C<IR, C=HP
JMin	254.2 ± 23.4	205.1 ± 118.8	IR=C	269.8 ± 23	103.5 ± 99.2	193.1 ± 127.9	IR⋘C, HP<C
JMax	229.7 ± 24.5	149.3 ± 119.1	IR<C	228 ± 24.9	151.8 ± 127.4	185.4 ± 74.7	IR=HP=C

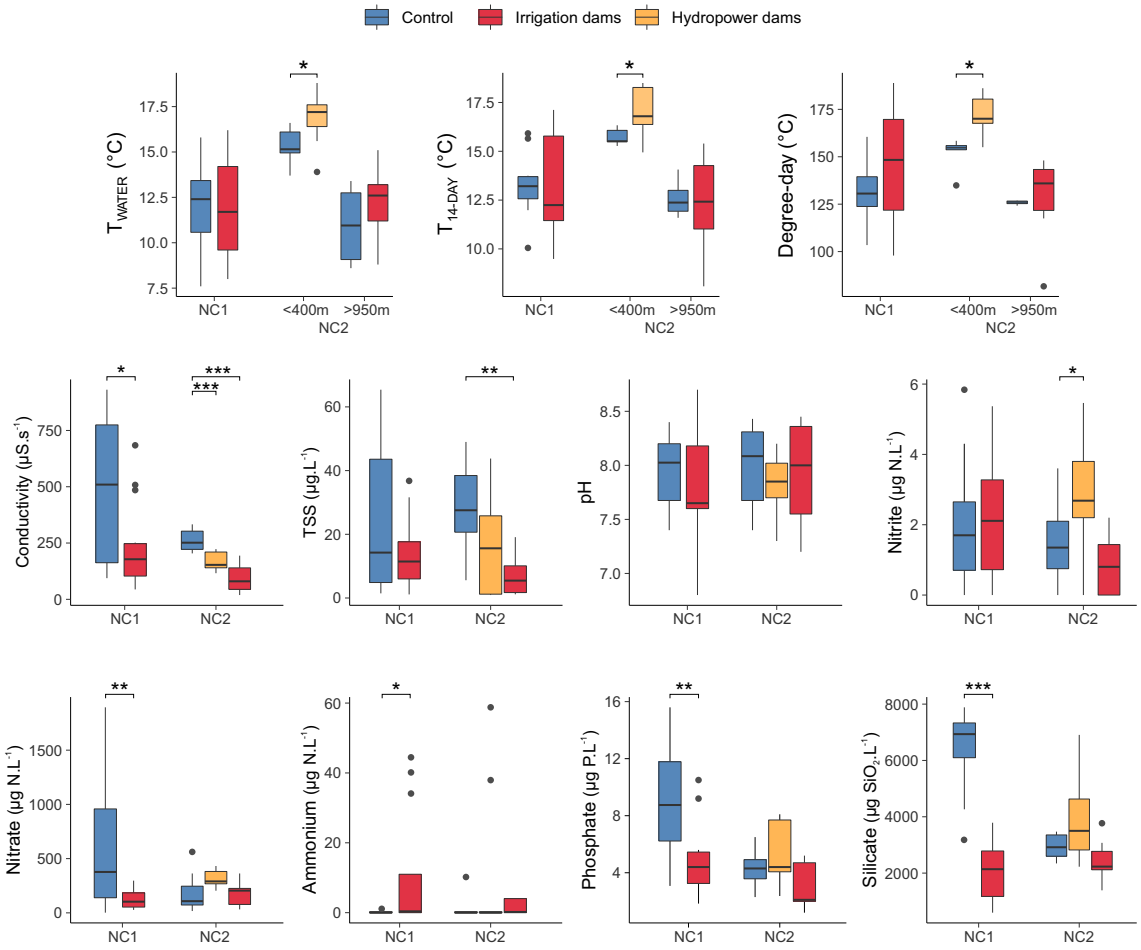


Figure 5.2 Boxplots representing water temperature, nutrients, and other water quality variables in NC1 (12 samples for controls, 16 for irrigation dams) and NC2 (12 samples for controls, 9 for irrigation dams, 9 for hydropower dams). Significant p-values (Tukey pairwise comparisons) are represented as $*** \leq 0.001, ** \leq 0.01, * \leq 0.05$.

5.3.2 Dam effects on biotic indices

Mean values of diatom biotic indices (Figure 5.3a) increased in most of the impacted sites; however, only the taxonomic richness downstream of hydropower dams had a significant increase in comparison to controls. Nitrite, silicate, and water temperature were significantly correlated to the biotic indices in NC1 (Figure S5.3a in Supplementary Material). In NC2, HIs and physico-chemical characteristics altered by irrigation dams (winter and summer flows, duration of high flow pulses, pH, silicate, and water temperature) showed significant correlations to the taxonomic richness and IPS score (Figure S5.3b).

The macroinvertebrate taxonomic richness and the Shannon-Wiener diversity index (Figure 5.3b) tended to decrease in impacted sites in comparison to controls, with significant differences downstream of irrigation dams in NC2 only. In general, they were significantly linked to HIs altered by irrigation dams (e.g. winter and

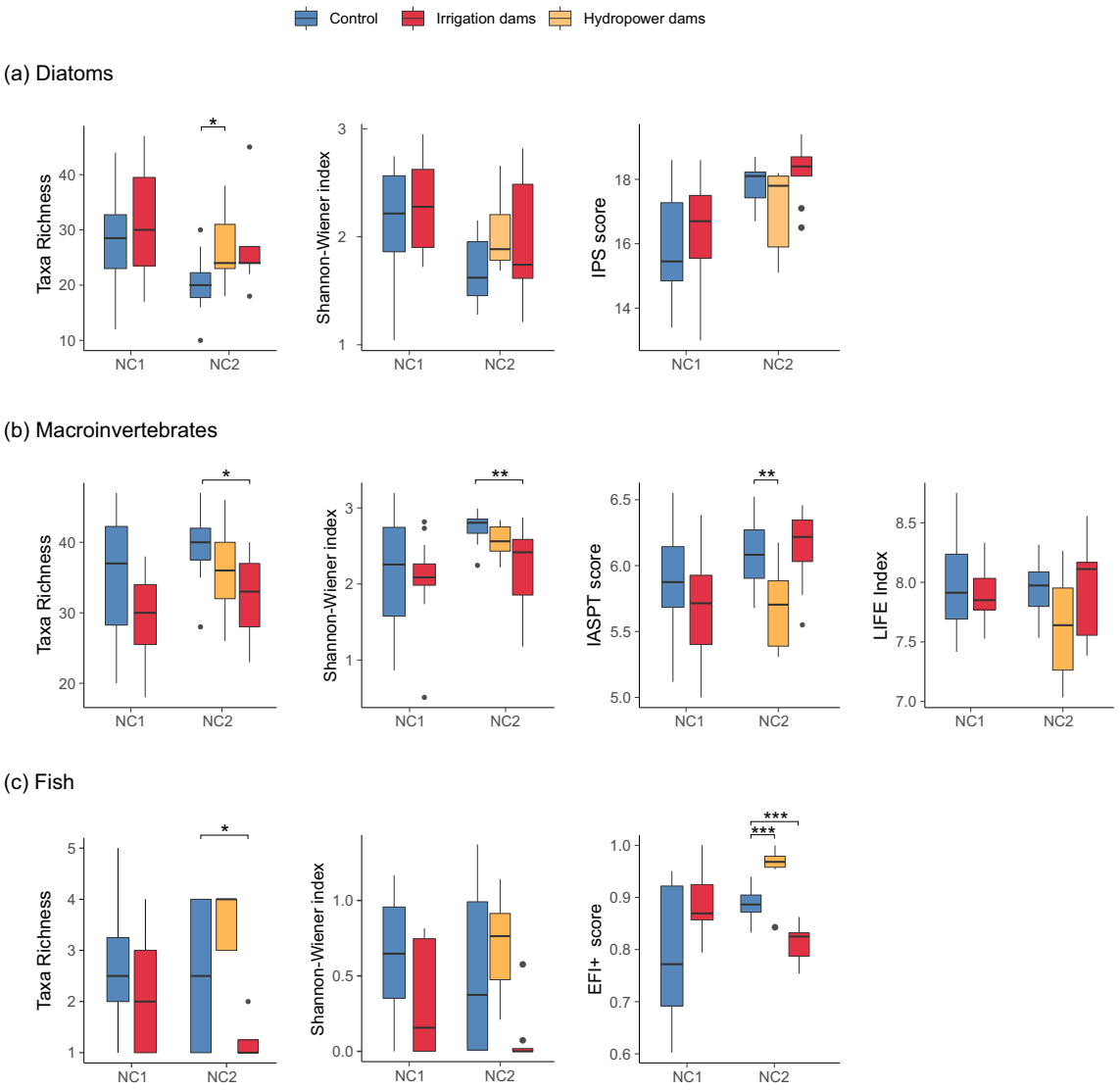


Figure 5.3 Boxplots representing taxonomic richness, Shannon-Wiener diversity index, and biotic indices of (a) diatoms, (b) macroinvertebrates, and (c) fish in NC1 (12 samples for controls, 16 for irrigation dams) and NC2 (12 samples for controls, 9 for irrigation dams, 9 for hydropower dams). Significant p-values (Tukey pairwise comparisons) are represented as $*** \leq 0.001, ** \leq 0.01, * \leq 0.05$.

summer flows, low flow extremes, number of flow increases and decreases) and by water temperature in NC1 (Figure S5.3a in Supplementary Material). The LIFE and IASPT mean scores also tended to decrease in most altered sites, although only significant differences in IASPT score were detected downstream of hydropower dams (Figure 5.3b). The indices showed few significant links to HIs and water quality in NC1, while in NC2, the IASPT score was significantly correlated to summer flows, seasonal low flow extremes, number and duration of annual low flow pulses, and water temperature (Figure S5.3b).

Fish communities (Figure 5.3c) in irrigation-impacted streams showed lower mean taxonomic richness and diversity in relation to controls; however, significant

changes in taxonomic richness were observed downstream of irrigation dams in NC2 only. The fish taxonomic richness and diversity were significantly related to HIs and water quality variables altered by irrigation and hydropower dams (winter flows, flow change rates, high flows frequency, TSS, water conductivity, and temperature; Figure S5.3 in Supplementary Material) in both hydrological classes. The EFI+ score showed significant changes in NC2, with increases downstream of hydropower dams and decreases in irrigation dams. This score was significantly related to monthly flows and physico-chemical variables in NC1 and strongly influenced by irrigation- and hydropower-altered variables (e.g. summer and winter flows, high and low flow events, nutrient concentrations, water temperature) in NC2 (Figure S5.3).

5.3.3 Trait-based community responses to flow regulation

5.3.3.1 Diatoms

The overall RLQ permutation test showed a non-significant trait-environment relationship (permutation across sites p=0.017, across species p=0.258) in NC1. The first two axes of the RLQ analysis explained 69.7% of the cross variance between diatom traits and the environment (Figure 5.4a-d). Along the first axis (42.7%), sampling sites were segregated according to flow regulation. The planktonic guild and larger diatom cell sizes (+Biovolume) were significantly associated to the magnitude of daily to seasonal low flow extremes (+1-90LF; see fourth-corner analysis in Figure S5.4 in Supplementary Material) and a low number of flow decreases (-nNeg), and stalked diatoms showed significant associations to lower winter flows (-M1), less negative flow fall rates (+meanNeg), and lower TSS and nitrite concentrations (variables altered by irrigation dams in our study). In contrast, motile and adnate diatoms were associated to hydrological characteristics found in control sites (e.g. -90LF, -meanNeg).

In NC2, the overall RLQ trait-environment link was marginally significant (permutation across sites p<0.001, across species p=0.072). The two first axes of the RLQ analysis (Figure 5.4e-h) explained 91.1% of the cross variance between diatom traits and environmental variables. Along the first axis (50.2%), samples were segregated according to the flow regulation, with exception of the Nansa samples (NAN17, NAN19), which overlapped the control group. However, most of the trait-environment relationships were concentrated in the second RLQ axis (40.9%). Along this axis, planktonic diatoms showed significant positive relationships with autumn flows (+M9-10), the magnitude of daily low flow extremes (+1LF), and the mean duration of high flow pulses (+meanDPHigh; see fourth-corner analysis in Figure S5.4 in Supplementary Material), i.e. HIs altered by irrigation dams in

NC2. Larger diatom cell sizes, low profile, adnate, and motile diatoms were, in turn, significantly linked to hydrological and physico-chemical conditions found in controls and hydropower dams (e.g. lower summer and autumn flows, stronger flow changes, higher water temperature and nutrient concentrations). In addition, the position of controls and hydropower-impacted sites along the second axis was related to the hydrological year of the survey: samples collected in 2017 (dry year), 2019 (average year), and 2018 (wet year). This temporal pattern could be linked to a gradient of increasing flows (+meanPos, +nPulsesHigh) in 2018 samples in comparison to 2017 ones. In irrigation-impacted sites, however, this pattern was not clear.

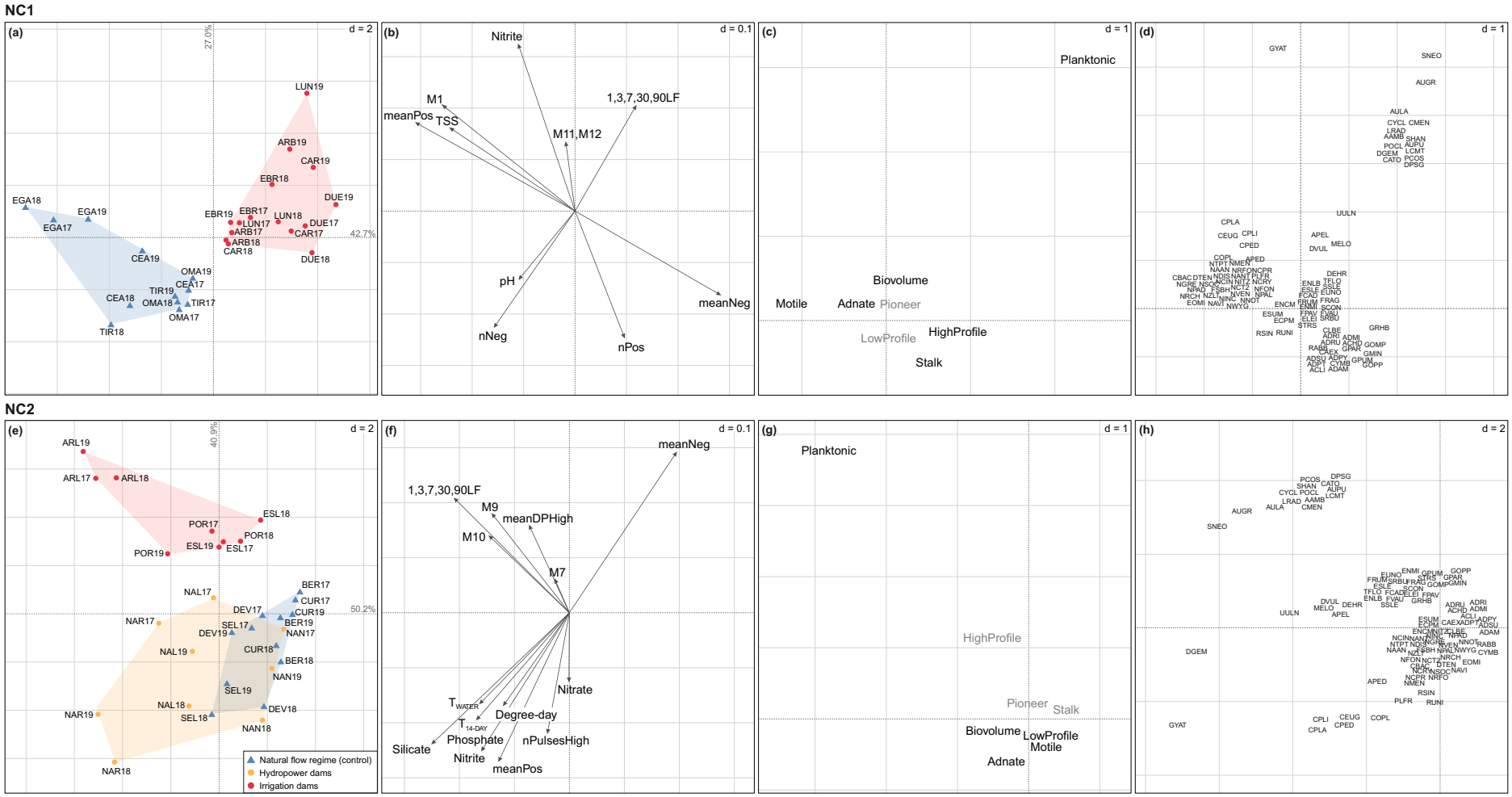


Figure 5.4 Ordinations showing the first two RLQ axes on diatom communities in NC1 (top) and NC2 (bottom). (a, e) Sampling site scores according to flow regulation (site code and year of sampling, see Table 5.1). (b, f) Coefficients for key HIs and local environmental variables (see Table A.1 for codes) with strong links to traits. (c, g) Coefficients for traits (see Table B.1 for codes). Traits with no significant relationships (see fourth-corner analysis, Figure S5.4) are represented in grey. (d, h) Coefficients for species (see Table S5.1). The "d" values give the grid size for scale comparison across graphs.

5.3.3.2 Macroinvertebrates

The overall RLQ permutation test showed a significant macroinvertebrate trait-environment relationship (permutation across sites $p<0.001$, across species $p=0.018$) in NC1. The first two RLQ axes accounted for 93.2% of the total variance (Figure 5.5a-d), with the first axis (86.8%) segregating control from impacted sites. Along this axis, macroinvertebrates with longer life cycles (Perennial), medium to large body sizes (MedLarge; >2-4cm), adult aquatic stages (Adult), ovoviviparous, and shredders showed positive significant associations to hydrological and physico-chemical conditions found in NC1 control sites (e.g. increased winter flows and nutrient concentrations, higher magnitude and frequency of high flow events; see the fourth-corner analysis in Figure S5.5 in Supplementary Material). These traits were replaced by macroinvertebrates with shorter life cycles (Ephem; life cycles $\leq$ 1 year), small to medium body sizes (SmallMed; >0.5-1cm), deposit feeders (DepositFeeder), and taxa with eggs resistant to unfavourable conditions (DormantEggs) in sites marked by the inversion of seasonal flow patterns caused by irrigation dams (e.g. +M6-10, +90LF, +Reversals). The second axis (6.4%) was mainly linked to temperature and voltinism. Higher water temperatures ($+T_{14\text{-DAY}}$, +Degree-day), higher number of days with increasing flows (+nPos), and lower autumn flows (+M11) were significantly related to taxa with multiple reproductive cycles per year (Multivoltine), replacing crawlers, univoltine, and semivoltine taxa in streams with lower temperature.

In NC2 (Figure 5.5e-h), the overall RLQ trait-environmental link was marginally significant (permutation across sites $p<0.001$, across species $p=0.072$). Along the first axis (65.3%), macroinvertebrates with longer life cycles (Perennial), flyers, and gill respiration were significantly associated to unregulated flow conditions in NC2 (e.g. increased winter flows, more frequent high flow events, later timing of the annual minimum flows, and higher nutrient concentrations; see the fourth-corner analysis in Figure S5.6 in Supplementary Material). In contrast, sites impacted by irrigation dams were concentrated in the opposite direction, favouring burrowers, endobenthic organisms (Interstitial), tegument respiration, taxa with resistance strategies to unfavourable conditions (Cocoons), and asexual reproduction (Asexual). Along the second axis (11.4%), conditions characterising unregulated streams (e.g. +M11, +1-3HF, +JMin, +nNeg, +TSS) were significantly related to perennial and univoltine taxa. They were replaced by multivoltine taxa, aquatic passive dissemination (AquPassive), and scrapers in streams affected by hydropower dams (e.g. higher water temperatures, increased spring flows).

In addition, a gradient from the driest (2017 samples towards the upper part; Figure 5.5e) to the most humid year (2018 samples towards the bottom) was present along the second axis in NC2. As such, univoltine and semivoltine taxa, medium body sizes (Medium; $> 1-2$cm), and cemented isolated eggs (IsolEggsFixed) tended to increase with more frequent flow decreases (+nNeg), less negative fall rates (+meanNeg), and higher November flows (+M11) toward the upper part of the graph. They were replaced by multivoltine, ovoviviparous taxa, aquatic passive dissemination, small to medium body sizes (SmallMed; $> 0.5-1$cm), and filter feeders and were, in turn, associated to higher water temperatures, increased nitrite and nitrate concentrations, higher magnitude and frequency of high flows (+30-90HF, +FRE7, +nPos), and higher spring flows (+M4-6).

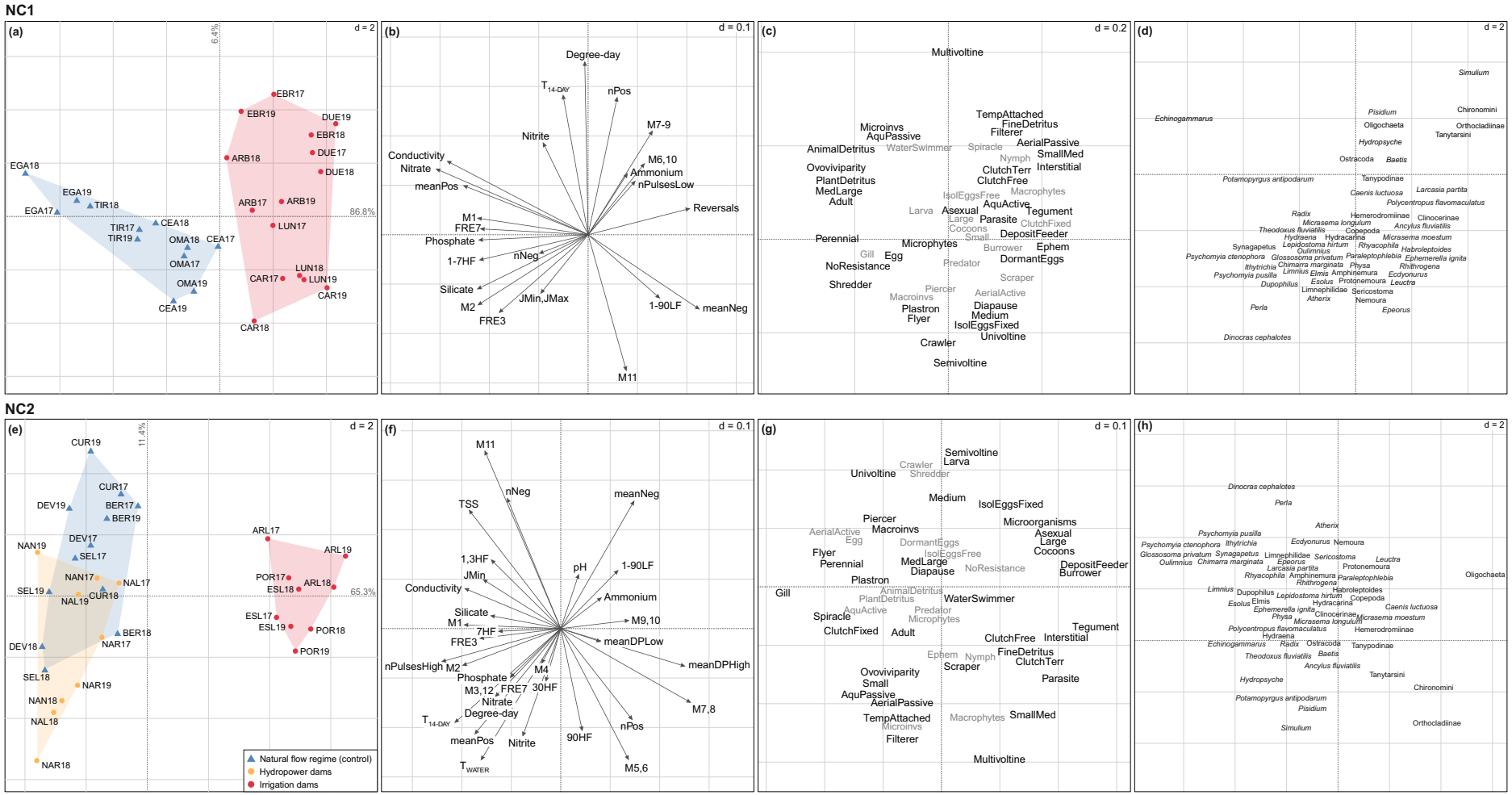


Figure 5.5 Ordinations showing the first two RLQ axes on macroinvertebrate communities in NC1 (top) and NC2 (bottom). (a, e) Sampling site scores according to flow regulation (site code and year of sampling, see Table 5.1); (b, f) coefficients for HIs and environmental variables with strong links to traits (see Table A.1 for codes). (c, g) Coefficients for functional traits (see Table B.2 for codes). Traits with no significant relationships (fourth-corner analysis, Figure S5.5 - S5.6) are represented in grey. (d, h) Coefficients for species, see Table S5.2. The "d" values give the grid size for scale comparison across graphs.

5.3.3.3 Fish

In NC1, the overall trait-environment link was marginally significant (permutation across sites p=0.007, across species p=0.065). The first two axes of the RLQ analysis (Figure 5.6a-d) explained 96.9% of the total co-inertia linking fish traits and the environment. Most of this variability was concentrated in the first axis (87.3%), which represented a gradient of fish migration and flow change. Non-migratory species (e.g. *Phoxinus bigerri*, *Achondrostoma arcasii*) tended to be more abundant with higher flow rise rates (+meanPos), while potamodromous and piscivorous fish (e.g. *Salmo trutta*) were linked to less negative fall rates (+meanNeg significantly linked to Piscivory only; see fourth-corner analysis in Figure S5.7). Across a gradient of flow regulation, benthic fish (e.g. *Barbatula quignardi*, *Gobio lozanoi*, *Cobitis calderoni*) were significantly linked to higher TSS concentrations, higher magnitude of daily floods (+1HF), and more frequent high flow events (+FRE3-7), which were conditions found in control sites. In contrast, species feeding at the water column (WaterColumn; e.g. *P. bigerri*) were significantly favoured by lower TSS concentrations and less frequent high flow events (-FRE7), that is, conditions found in streams affected by irrigation dams. Moreover, omnivorous fish species (e.g. *P. bigerri*) were significantly associated to increasing water temperatures (+Degree-day) but replaced by invertivorous ones at lower temperatures.

In NC2, the overall trait-environment link was non-significant (permutation across sites p<0.001, across species p=0.148). The first two axes of the RLQ analysis (Figure 5.6e-h) explained 98.6% of the total co-inertia linking fish traits and the environment. Along the first RLQ axis (92.9%), samples were segregated according to flow regulation and dam purpose, with exception of the 2019 Sella sample (SEL19), which overlapped the hydropower-impacted group. In sites affected by irrigation dams (+M7-8, +meanDPHigh, +meanNeg), species feeding at the water column (e.g. *S. trutta*) and potamodromous fish (e.g. *S. trutta*) were significantly favoured. Species performing long-distance migrations (LongMigration, e.g. *S. salar*) and large-sized fish (MaxLength, e.g. *Anguilla anguilla*) seemed to be favoured by increasing flows (+meanPos, +nPulsesHigh) and higher silicate and nitrate concentrations, i.e. conditions found in control sites; however, these relationships were non-significant (see fourth-corner analysis in Figure S5.7). Hydrological and physico-chemical conditions characterising sites affected by hydropower dams did not show positive associations to fish traits. Furthermore, in contrast to the diatom and macroinvertebrate ordinations, the interannual variation was unclear in fish communities.

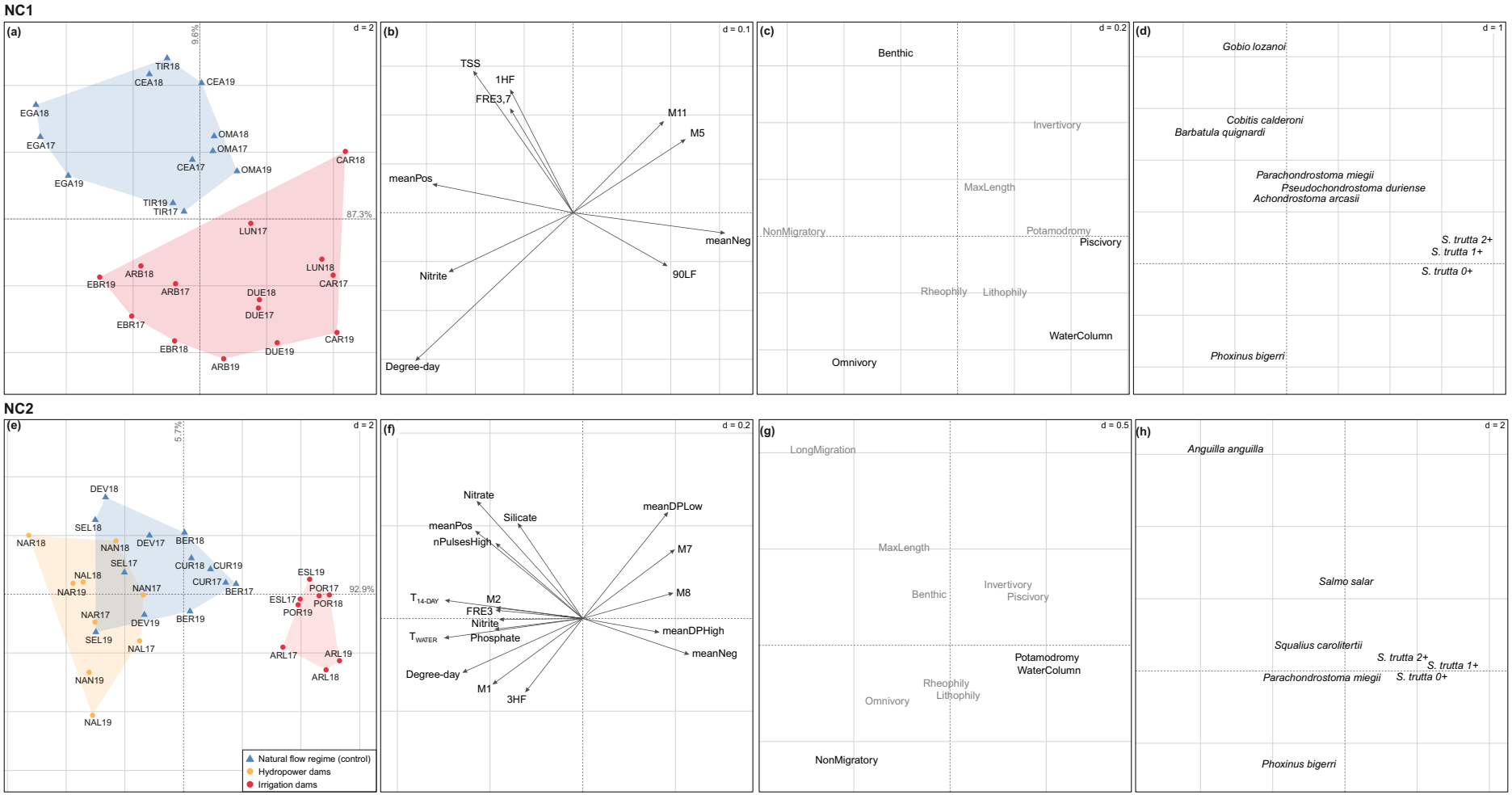


Figure 5.6 Ordinations showing the first two RLQ axes on fish communities in NC1 (top) and NC2 (bottom). (a, e) Sampling site scores according to flow regulation (site code and year of sampling, see Table 5.1). (b, f) Coefficients for key HIs and physico-chemical variables with strong links to traits (see Table A.1 for codes). (c, g) Coefficients for traits (see Table B.3 for codes). Traits with no significant relationships (see fourth-corner analysis, Figure S5.7) are represented in grey. (d, h) Coefficients for species, see Table S5.3. The "d" values give the grid size for scale comparison across graphs.

5.4 Discussion

The combination of a control-impact design and three consecutive year surveys covering multiple biotic groups allowed us to identify key ecological responses to flow regime changes associated to different dam purposes. As hypothesised, the different dam operation schemes generated different biophysical responses in rivers. Irrigation dams were associated to the inversion of seasonal flow patterns and to a reduction in nutrient concentrations, while hydropower dams were linked to higher rates of flow change and to increases in water temperature. Our second hypothesis also holds, as biological responses were community- and factor intensity-dependant. In this regard, it is worth noting that the taxonomic metrics were not always sensitive to dam impacts, while key trait-based community shifts emerged along the gradients of hydrological and physico-chemical dam-induced changes. Hydrological alteration (e.g. changes in low and high flow extremes, flow rise and fall rates, summer and winter flows, frequency and duration of low or high flow pulses), along with changes in water temperature and nutrient concentrations, showed significant relationships with trait-based community shifts, especially for macroinvertebrates and diatoms. Furthermore, dam-related impacts favoured a higher abundance of disturbance-tolerant traits (e.g. planktonic diatoms, multivoltine macroinvertebrates, non-migratory fish), as expected.

5.4.1 Environmental changes and dam purpose

Dam operational schemes (i.e. irrigation and hydropower) generated different changes in the hydrological and physico-chemical characteristics. Contrary to this result, Peñas and Barquín (2019) highlighted a lack of dependency between the hydrological alteration output and the purpose of the dam. Nonetheless, this discrepancy might relate to the contrasting spatial extent of both studies, as Peñas and Barquín (2019) covered the Iberian Peninsula and nine different hydrological classes. We found that river reaches affected by irrigation dams in both NC1 and NC2 showed increases in the summer flow magnitude and decreases in winter flows and in the frequency and magnitude of extreme high flow events. This result agreed with the observed inversion of river flow regimes to attend to urban and agricultural water demands (Richter and Thomas, 2007), which was also reported from Mediterranean rivers (García de Jalón et al., 2019).

The three dams used exclusively for hydropower generation (Narcea, Nalón, and Nansa) are located in Cantabric catchments, a region characterised by the confluence of coastal mountains and high precipitation levels (Esteban-Parra et al.,

2003). They have relatively lower storage indices in comparison to other reservoirs included in this study (on average, 1.7 times smaller; Table 5.1) and their hydrological impact was mostly related to the frequent day-to-day or even sub-daily flow changes (Zimmerman et al., 2010). Sub-daily discharge information (unpublished data; CHC, 2022) indicated abrupt flow changes downstream of Narcea dam (hourly discharges), while Nalón (weekly discharges) and Nansa (run-of-river operation) had less pronounced short-term hydrological alteration. In agreement with previous studies (e.g. Kuriqi et al., 2021; van Looy et al., 2007) our study found significant impacts on aquatic ecosystems produced by hydropower dams. Moreover, biological responses to these flow regime alterations were more clear in macroinvertebrate and diatom communities (see Figures 5.5e and 5.6e), as Narcea samples presented the largest deviation from the control group, while the Nansa ones showed to be more similar to controls. This result has potential implications when analysing biological responses to dam operation schemes, as hydropower dams can present drastic short-term flow changes caused by energy demand fluctuations (e.g. flood peaks occurring at the sub-daily or sub-hourly scale; Zimmerman et al., 2010). In this regard, finer-resolution hydrological information (e.g. hourly discharge) could be useful to unveil flow-ecology relationships that might not be captured when using daily hydrological datasets (Bevelhimer et al., 2015; Zimmerman et al., 2010).

Changes in water physico-chemical variables also varied with dam operational schemes, as also found elsewhere (Aristi et al., 2014; Kamidis et al., 2021; Ling et al., 2016). Irrigation dams considerably reduced the nutrient and TSS concentrations downstream in our study area. Friedl and Wüest (2002) reported that the large storage volumes and extended residence times of irrigation dams are capable of retaining nutrients and suspended solids, causing disturbances in ecosystem structure and functioning downstream. Romero et al. (2016) gave further evidence on the effect of reservoirs with large regulatory capacity in Mediterranean climates, with nitrogen retention rates up to 90%. Hydropower dams presented significantly higher water temperatures in comparison to controls located at similar altitudes. Unlike large reservoirs, small dams present little or no thermal stratification, and water temperature downstream tends to rise due to the surface exposure to solar radiation, low flow velocities, and low magnitudes of short-term minimum flows (Bejarano et al., 2017; Chandesris et al., 2019). Therefore, the dam's operational schemes can have a significant impact on how they affect geophysical processes and river biota.

5.4.2 Biotic indices responses to irrigation and hydropower dams

In general, biotic indices responses varied across dam types and natural classes. Diatom indices presented slightly higher scores in altered rivers and showed significant correlations to physico-chemical variables. Recent studies have pointed out similar results, for instance, Krajenbrink et al. (2019) found that the Trophic Diatom Index scored higher at impacted sites. These findings indicate a possible community shift downstream of irrigation dams to adapt to lower nutrient and TSS concentrations and to more stable and permanent flows, conditions that have been shown to favour the development and growth of biofilm algae (Biggs et al., 1998). The reduction of grazers downstream of dams also contributes to a primary producer community shift towards more diverse communities (Liess and Hillebrand, 2004). Literature on diatom taxa richness below dams is relatively scarce, but most research to date has reported a greater number of taxa at dam downstream sites (Growns and Growns, 2001; Smolar-Žvanut and Mikoš, 2014), similar to the results found in our study. However, our results indicated that the direction of community change would highly depend on which direction hydrological and water physicochemical characteristics are altered.

Macroinvertebrate richness and diversity showed important responses to HIs and physico-chemical variables significantly altered by the presence of irrigation dams (e.g. summer flows, low flow extremes, frequency of high flow events, nutrient concentrations). These results suggest that these biotic indices may be good indicators of the biological impacts caused by these dams, although they have been rarely used for this purpose (but see Holt et al., 2015). In contrast, IASPT and LIFE scores showed weak responses to HIs and water quality variables altered by dams. This is not surprising since most biotic indices were originally designed to target organic pollution and water quality rather than other stressors (Bonada et al., 2006; Friberg et al., 2011). Conversely, the LIFE index, a macroinvertebrate metric designed to evaluate hydromorphological alteration (Extence et al., 1999), failed to detect the effects of irrigation dams on macroinvertebrate communities. Similar results have also been reported previously (e.g. Álvarez-Cabria et al., 2017; Laini et al., 2018). This limitation might be associated with different hydrological preferences of macroinvertebrates in Spanish streams and the effect of other relevant variables such as temperature and water quality (Armanini et al., 2011; Laini et al., 2018).

In relation to fish, it is important to remark that the studied communities were species-poor (less than four species in any case). In fact, in NC2 streams impacted by irrigation dams, communities were composed almost exclusively of brown trout

(Table S5.3 in Supplementary Material), decreasing taxonomic richness, diversity, and EFI+ scores (Figure 5.3). In contrast, downstream of hydropower dams, the presence of Atlantic salmon and brown trout (i.e. salmonid species considered intolerant to oxygen depletion and habitat degradation) contributed substantially to increasing EFI+ index scores. Thus, despite the significant changes found between dam types for taxa richness and EFI+ scores, we believe that these results are not consistent with the dam hydrological or water physicochemical changes. In contrast, we believe these results are more related to biogeographical and dispersion-connectivity issues related to metapopulational dynamics of few species, as it has been demonstrated in nearby catchments for brown trout and transversal obstacles (González-Ferreras et al., 2019, 2022). In fact, fish communities in poor-taxa rivers might not be a good indicator of changes in niche characteristics, as it has been pointed out in other studies (Colin et al., 2018; Maire et al., 2015; Vogel et al., 2019).

5.4.3 Trait-based responses to irrigation and hydropower dams

Most diatom community shifts in NC1 and NC2 were linked to dam-related changes in the flow regime and physico-chemical conditions (Figure 5.4). Small-sized and low profile diatoms (adnate and pedunculate life forms, mostly represented in our dataset by *Cocconeis euglypta* and *Achnathidium pyrenaicum*, respectively) were replaced by planktonic and large-sized diatom cells in sites affected by irrigation dams, in agreement with Goldenberg-Vilar et al. (2021) and Wang et al. (2022). In NC2, the planktonic guild was linked to irrigation-impacted sites, while larger cell sizes and low profile adnate diatoms were more abundant in sites affected by hydropower dams. Planktonic diatoms are less resistant to high flow velocities and usually are found in higher numbers in lentic habitats (Rimet and Bouchez, 2012). Therefore, the more stable medium to low-flow conditions downstream irrigation impoundments may have facilitated the development of planktonic diatom assemblages (Growns, 1999; Krajenbrink et al., 2019; Petts, 1984). Adnate life forms are attached to the substrate by the whole valve area, i.e. adapted to the high flow velocities and scouring (Passy, 2007; Rimet and Bouchez, 2011; Wu et al., 2019b). Due to high flow daily disturbance in hydropower dams in comparison to controls, adnate life forms are found in higher numbers in these impacted sites, whereas low profile pedunculated forms dominate in controls. It must be highlighted that recent studies have pointed out contrasting relationships between the diatom traits and flow regimes (e.g. Dalu et al., 2017; Krajenbrink et al., 2019; Stenger-Kovács et al., 2013). This could be associated to the added effect of other factors, such as nutrient and/or sediment

loads. For instance, diatom size (biovolume), which showed contrasting results in NC1 and NC2, is a trait associated to nutrient availability but also to low flow velocities and lentic habitats. More research is needed to determine whether the responses of various diatom guilds are linked to increasing nutrient concentrations, hydrological conditions, or a combination of both, as it is yet unclear how traits are related to environmental factors (Lavoie et al., 2006).

As expected, macroinvertebrate traits presented clear responses to the presence of dams and, in general, flow regulation favoured disturbance-tolerant traits, as also seen elsewhere (Berger et al., 2018; Murphy et al., 2021). Maximum body sizes (small to medium sizes in NC1, larger sizes in NC2), life cycle duration ($\leq$ 1 year), respiration (tegument respiration in NC2), food and feeding habits (fine detritus feeders in NC1, deposit feeders in NC2), burrower and interstitial taxa in NC2 were favoured by hydrological and physico-chemical conditions altered by irrigation dams. In turn, macroinvertebrates with longer life cycles (>1 year), adult aquatic stage, and shredders were more abundant in natural streams. These results are aligned with previous works assessing the effect of hydrological alteration in Mediterranean streams. For instance, Belmar et al. (2019) detected the dominance of small-sized and multivoltine taxa and a decrease of shredders in regulated perennial rivers in Spanish Mediterranean rivers. Parallel, Mendoza-Lera et al. (2012) and González et al. (2013) also reported a lower abundance of shredders in communities below headwater dams, as the reservoirs retain leaf litter and reduce their food resources (i.e. detritus). Therefore, these traits might be considered key traits to assess the effects of irrigation dams on macroinvertebrate assemblages. Nonetheless, it must be pointed out that the response of the macroinvertebrate communities is also hydrological-class dependant as the association between shredders and flow regime or physico-chemical variables was only significant in NC1.

Although we found no significant variation in water temperature among controls and irrigation dams (i.e. the largest dams in our study), a thermal gradient in NC1 macroinvertebrate communities was significantly associated to voltinism, a trend also reported by Lawrence et al. (2010). Temperature plays a key role in organisms' growth and life cycle duration as their development depends on heat accumulation above a certain temperature threshold (Ward and Stanford, 1982; Woodward et al., 2010). These findings imply that macroinvertebrates might be particularly vulnerable to factors other than flow regulation, e.g. water temperature altered by dams (Chessman, 2009; Durance and Ormerod, 2007), causing important shifts in their life cycles and, as a consequence on macroinvertebrate community structure and composition.

The fish community shifts associated to hydrological and physico-chemical changes were less pronounced than in diatoms and macroinvertebrates (i.e. a lower number of significant trait-environment associations). As pointed out earlier, we believe that the limited number of fish species (and hence traits) inhabiting the studied streams might limit the sensitivity of fish communities to establish niche-related changes generated by dams (Colin et al., 2018; Maire et al., 2015; Vogel et al., 2019). However, it is important to notice that flow regulation favoured generalists (e.g. omnivorous, non-migratory species) over specialists (e.g. benthic, migratory species). For instance, the absence of benthic invertivorous fish (a sensitive trait, e.g. *Barbatula quignardi*) in impacted sites can be linked to changes in substrate and macroinvertebrate communities (i.e. their main food source) downstream of dams, as also reported by Granzotti et al. (2018).

5.4.4 Implications for dam management

The current high irrigation demands (around 45% of the total annual water consumption in Spain; Fornés et al., 2021) and the increasing future water needs worldwide aggravated by climate change (Törnqvist et al., 2015) underline the urgency of investigating dam impacts on freshwater ecosystems. Few of the biotic indices covered in our study showed significant responses to dams. Most of them have been designed to assess water quality in terms of eutrophication and organic pollution, highlighting the limitations of the available biomonitoring tools to assess hydrological alteration (Ladrera and Prat, 2013; Wang et al., 2022). Moreover, the lack of a mechanistic understanding between the change on this biotic index and the key factors altered by dams (e.g. temperature, water quality, flow regime) limits their use to inform future management actions (De Castro-Català et al., 2020).

In this context, trait-based multivariate analyses allowed to establish a better mechanistic understanding linking key traits and main environmental changes induced by dams and, therefore, this approach could provide a better conceptual framework to generate more accurate prescriptions for water managers (e.g. Figure 5.7). In this regard, macroinvertebrate communities showed the strongest trait-based responses to irrigation and hydropower dams (i.e. more significant associations between traits and variables altered by these dams), followed by diatoms and fish. In hydropower dams, these responses were less evident, and fish traits did not show significant associations to hydropower-altered variables. Although the relationships identified in this study do not necessarily reflect direct cause-effect relationships, our findings emphasise the need to acknowledge the links between ecological components (e.g. multiple biological communities, ecosystem functions) and the main dam-related drivers of

change (e.g. flow, temperature, water quality regimes). This conceptual framework might be very useful to develop effective biomonitoring programmes (e.g. identify sensitive traits to target specific impacts), conservation strategies (e.g. environmental flows or restoration of thermal and nutrient regimes), and to set future research priorities tackling dam impacts on river ecosystems.

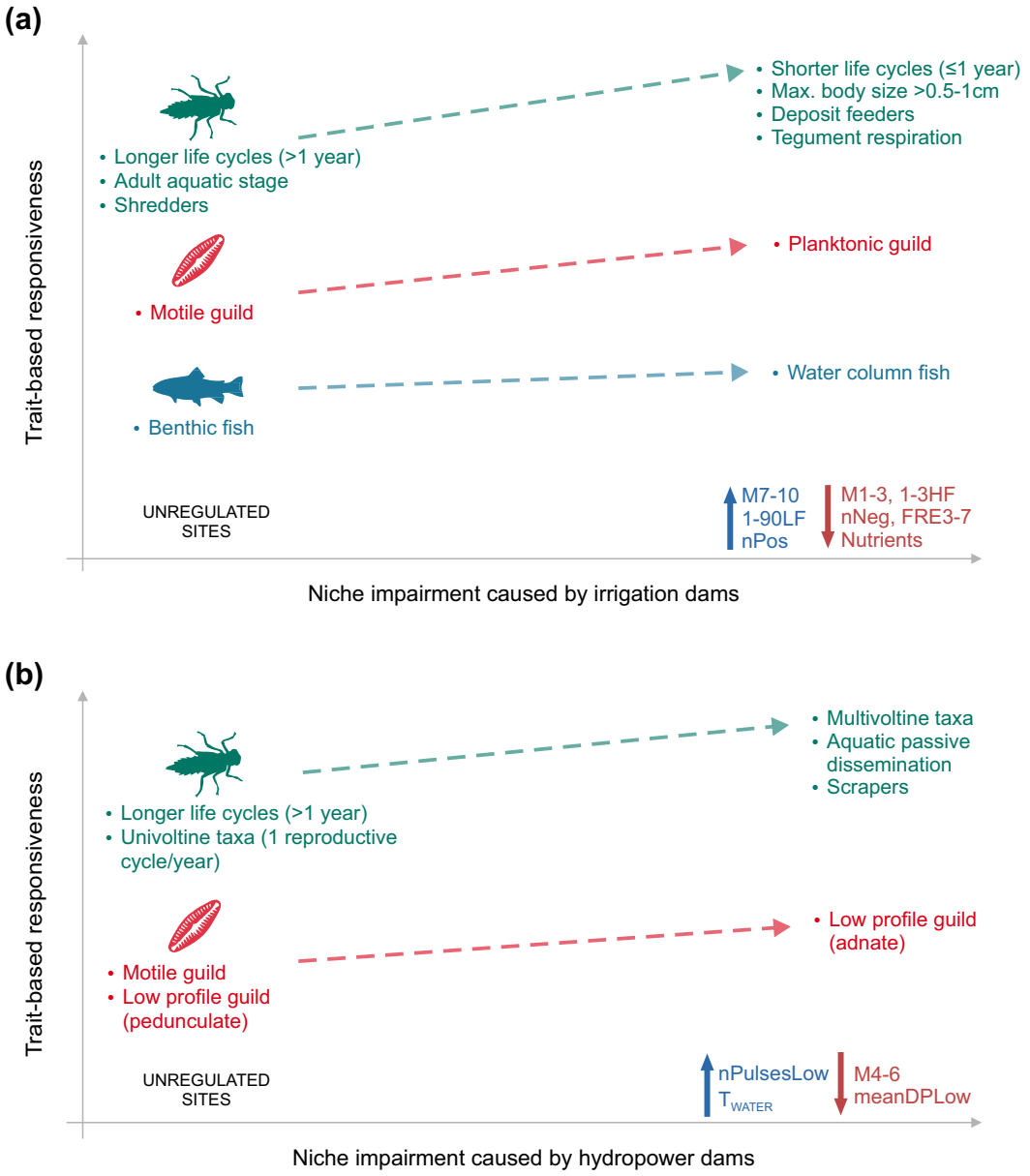


Figure 5.7 Main trait-based community shifts (higher abundances of traits) in diatoms (in red), macroinvertebrates (in green), and fish (in blue) associated to dam-induced changes detected in sites impacted by (a) irrigation and (b) hydropower dams.

Many environmental flow guidelines directed to diminish the ecological effects of dam operation schemes are designed worldwide using hydrobiological models, e.g. in Spain (Aguilar and Polo, 2016), Canada (Bradford et al., 2011), and Korea (Park et al., 2020). These models evaluate the habitat suitability for specific fish species as a function of species tolerance to a given set of habitat conditions (mainly

velocity, depth, and substrate; Poff et al., 2017). However, other factors (e.g. turbidity, nutrient concentration, water temperature, food availability, time of the day) might also impact species habitat selection, generating biased results (Armanini et al., 2011; Palau et al., 2016; Railsback, 2016). Furthermore, in species-poor rivers, the assessment of anthropogenic pressures is likely to be hampered when using methods sensitive to specific species, the presence/absence of rare taxa, and non-native species (Aparicio et al., 2011; Chen et al., 2020; Colin et al., 2018). In this context, as supported by our findings, macroinvertebrate and diatom communities, which are organisms ubiquitously found in rivers and with lower dependency on dispersal-connectivity (Shurin et al., 2009), could provide a more accurate biological information source for the assessment of dam impacts in terms of responsiveness to niche changes. These communities seem to be suitable to develop specific cause-effect relationships with the key environmental factors (e.g. HIs, water quality) that control changes downstream of dams and, thus, better support adaptations to dam operation schemes towards sustainability. Lastly, these key relationships can also guide future research priorities and monitor the effectiveness of environmental flows or restoration of other niche characteristics (e.g. thermal or nutrient regimes).

Finally, it is worth noting some of the study limitations. Firstly, the three-year period of sampling in this study does not embrace the entire potential interannual hydrological and physico-chemical variability within these sites, which might provide an incomplete understanding of the long-term effects of dams on these riverine ecosystems. Nonetheless, previous works covering the same study area have found similar hydrological patterns between this three-year series and the long-term period series (Goldenberg-Vilar et al., 2021). In addition, potentially relevant environmental factors (e.g. substrate composition, sub-daily flow changes) may be missing. Therefore, water agencies and dam operators should keep long-term monitoring programmes covering hydrological, physico-chemical, and biological sampling to disentangle the actual effect of dam operation schemes on river ecosystems, which could lead to an effective adaptive management cycle. Secondly, most of the results of this study and many others dealing with dam effects on river biota (e.g. Sánchez-Pérez et al., 2020; Wu et al., 2019b) are based on correlations between biological response and potential environmental predictors. Long-term and real-scale experiments would be a major breakthrough in this study field and could help investigate the impact of changes in dam operational schemes (Olden et al., 2014). Thus, we encourage setting up experiments in partnership with dam operators (e.g. Consoli et al., 2022) to validate key ecological-environmental responses that could orientate future efforts to reduce the negative effects of dams on river biodiversity.

In summary, our findings indicated that the seasonal flow regime inversion, nutrient reduction downstream of irrigation dams, the elevated rate of flow change, and water temperature associated to hydropower dams caused significant trait-based community shifts in macroinvertebrate communities, followed in intensity by diatoms and fish. In contrast to biotic indices, traits describing body size, life cycle duration, respiration, feeding habits, and ecological guilds allowed us to establish a better mechanistic understanding of the impacts of hydrological and physico-chemical changes. Our findings may help to provide prescriptions for water managers to design conservation programmes (e.g. environmental flows) that allow reducing the negative effects of dam operation; however, water agencies, national, and regional governments must boost long-term monitoring programs that allow increasing our knowledge on the effects of dam operation in river ecosystems.

5.5 Supplementary Material

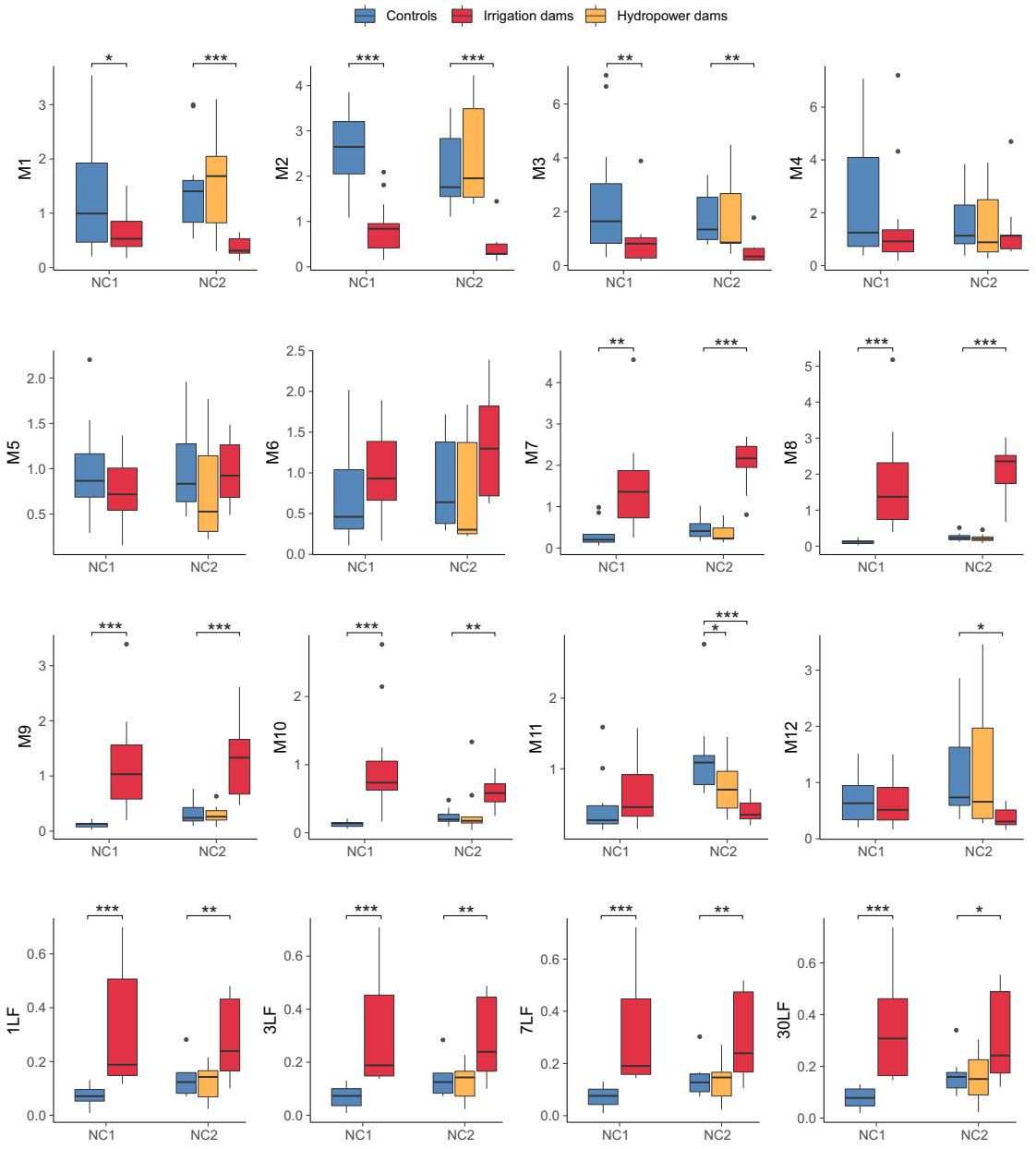


Figure S5.1 Boxplots of standardised hydrological indices in NC1: controls (n=12) and sites affected by irrigation dams (n=15); in NC2: controls (n=12), sites affected by hydropower (n=9), and by irrigation dams (n=9). See Table A.1 for hydrological indices abbreviations. Statistically significant differences (Tukey-pairwise comparisons) are represented as '***' $p \leq 0.001$, '**' $p \leq 0.01$, '*' $p \leq 0.05$.

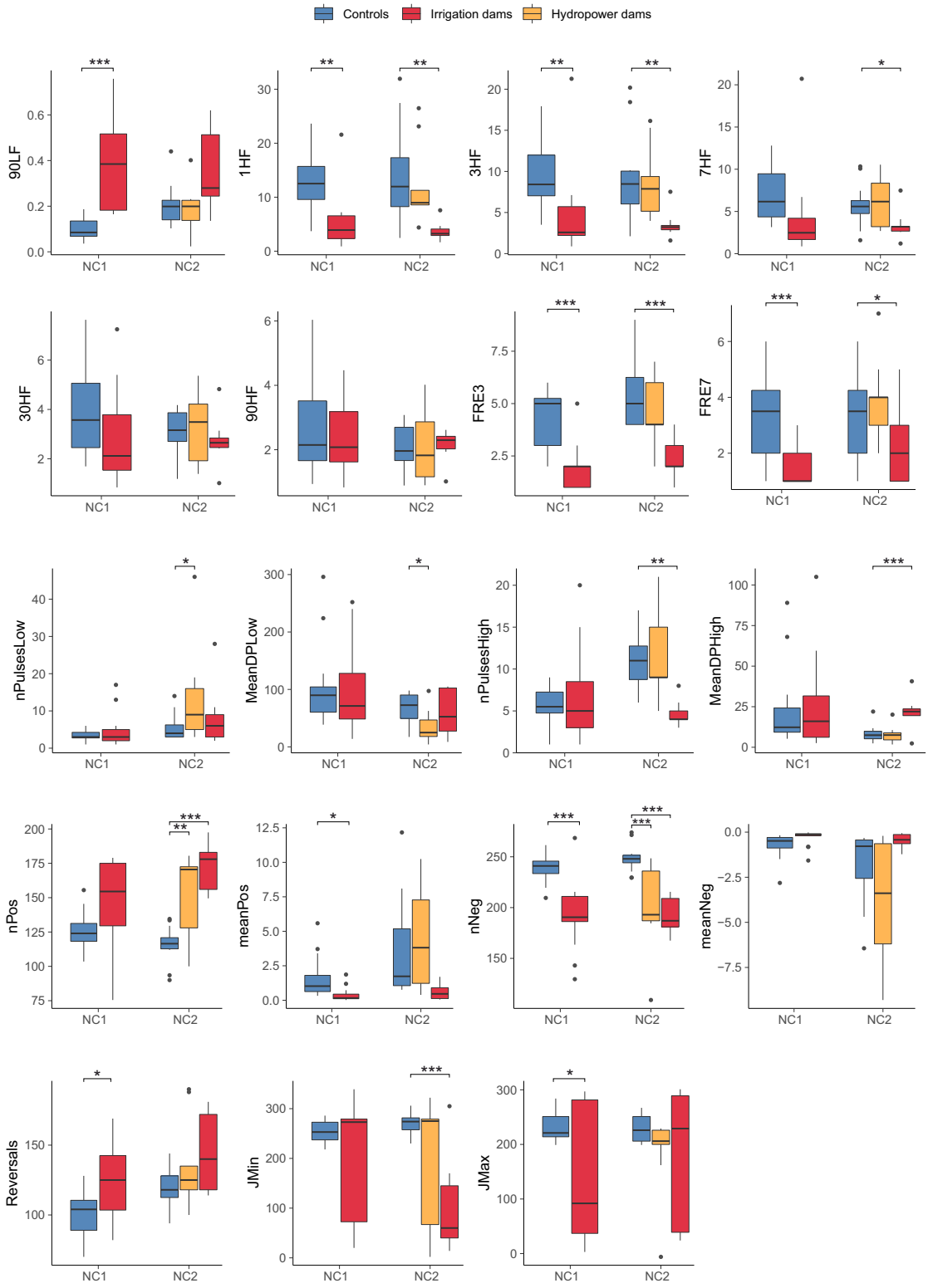


Figure S5.1 (cont.)

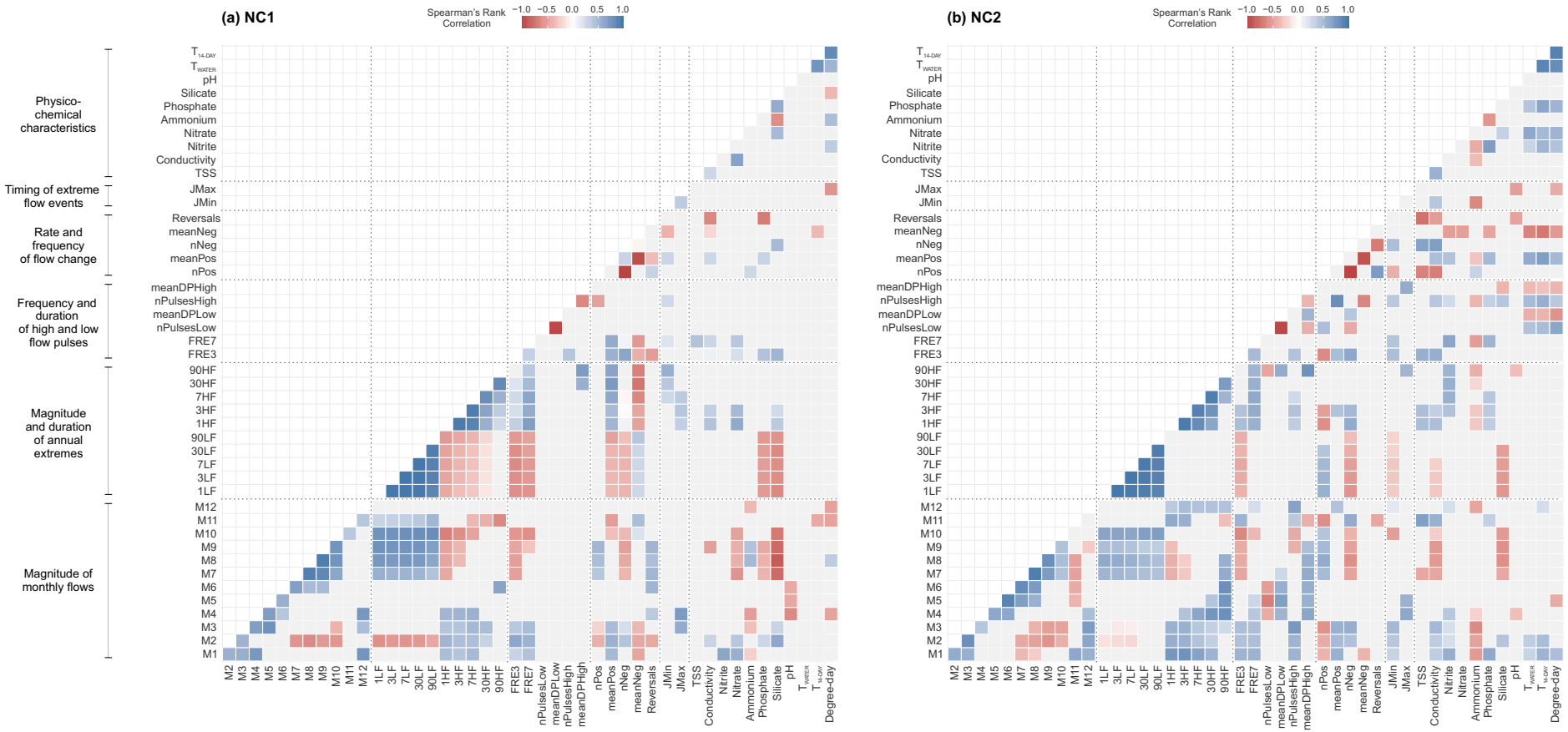


Figure S5.2 Correlation between biotic indices, hydrological, and physico-chemical variables in (a) NC1 and (b) NC2 streams assessed with Spearman's rank test. Grey boxes indicate non-significant associations (p>0.05).

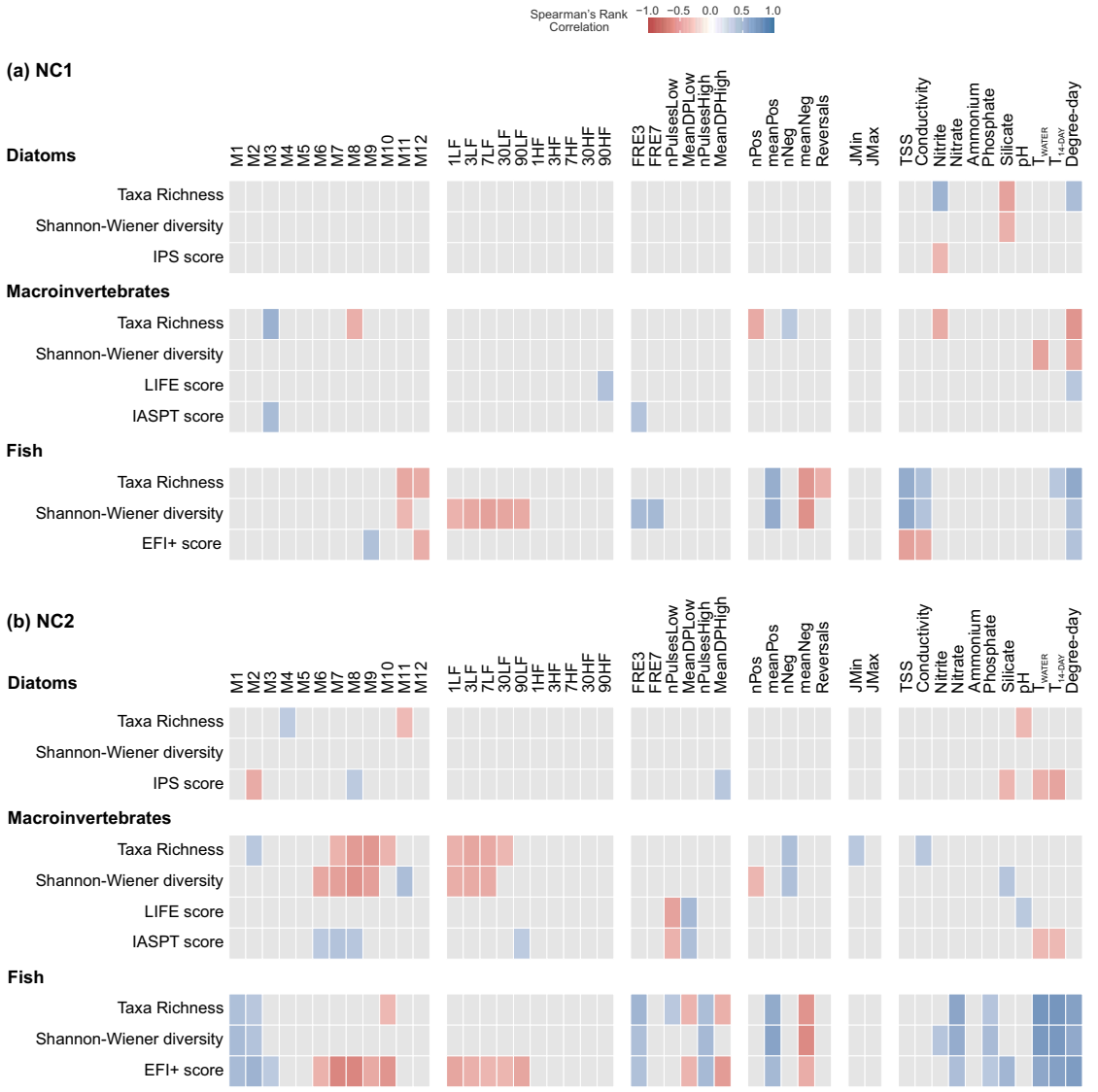


Figure S5.3 Correlation between hydrological and physico-chemical variables in (a) NC1 and (b) NC2 streams assessed with Spearman's rank test. Grey boxes indicate non-significant associations (p>0.05).

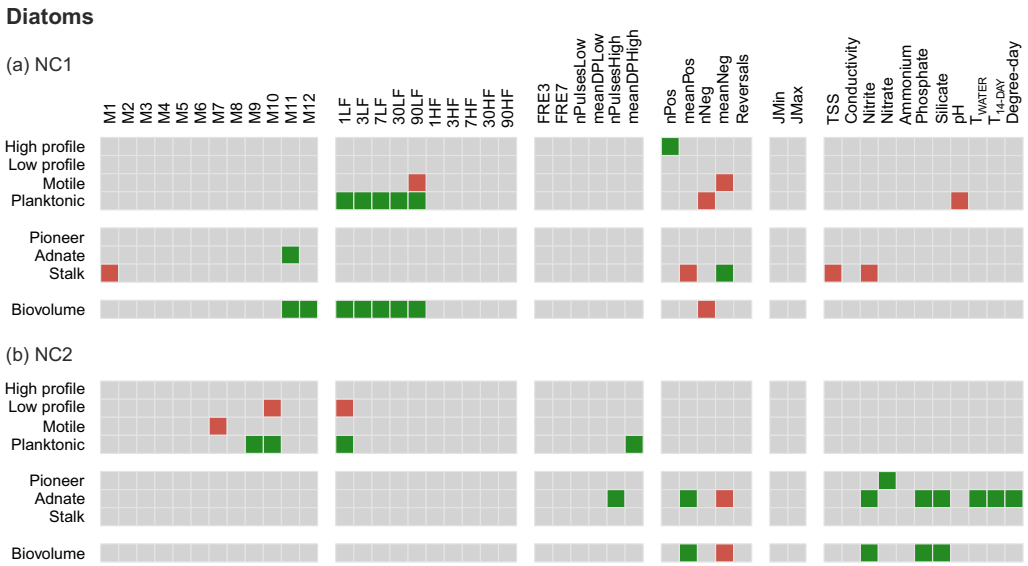


Figure S5.4 Fourth-corner correlations between diatoms traits (rows), hydrological (see Table A.1 for codes) and local physico-chemical variables (columns), in (a) NC1 and (b) NC2. Grey boxes indicate non-significant associations (p>0.05); red boxes indicate negative significant associations; green boxes indicate significant positive associations.



Figure S5.5 Fourth-corner correlations between macroinvertebrates traits (rows), hydrological (see Table A.1 for codes) and local physico-chemical variables (columns) in NC1 streams. Grey boxes indicate non-significant associations (p>0.05); red boxes indicate negative significant associations; green boxes indicate significant positive associations.

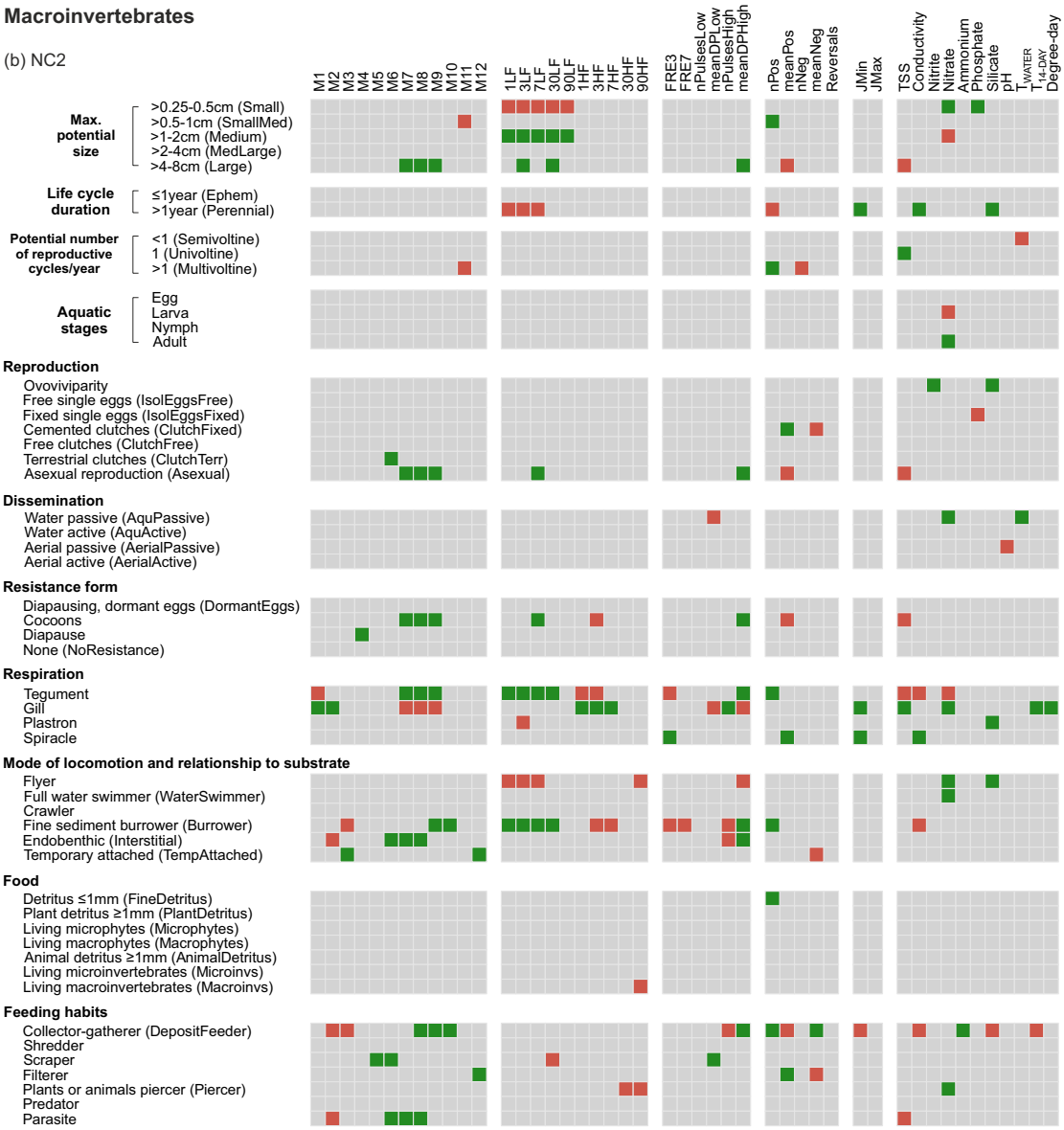


Figure S5.6 Fourth-corner correlations between macroinvertebrates traits (rows), hydrological (see Table A.1 for codes) and local physico-chemical variables (columns) in NC2 streams. Grey boxes indicate non-significant associations (p>0.05); red boxes indicate negative significant associations; green boxes indicate significant positive associations.

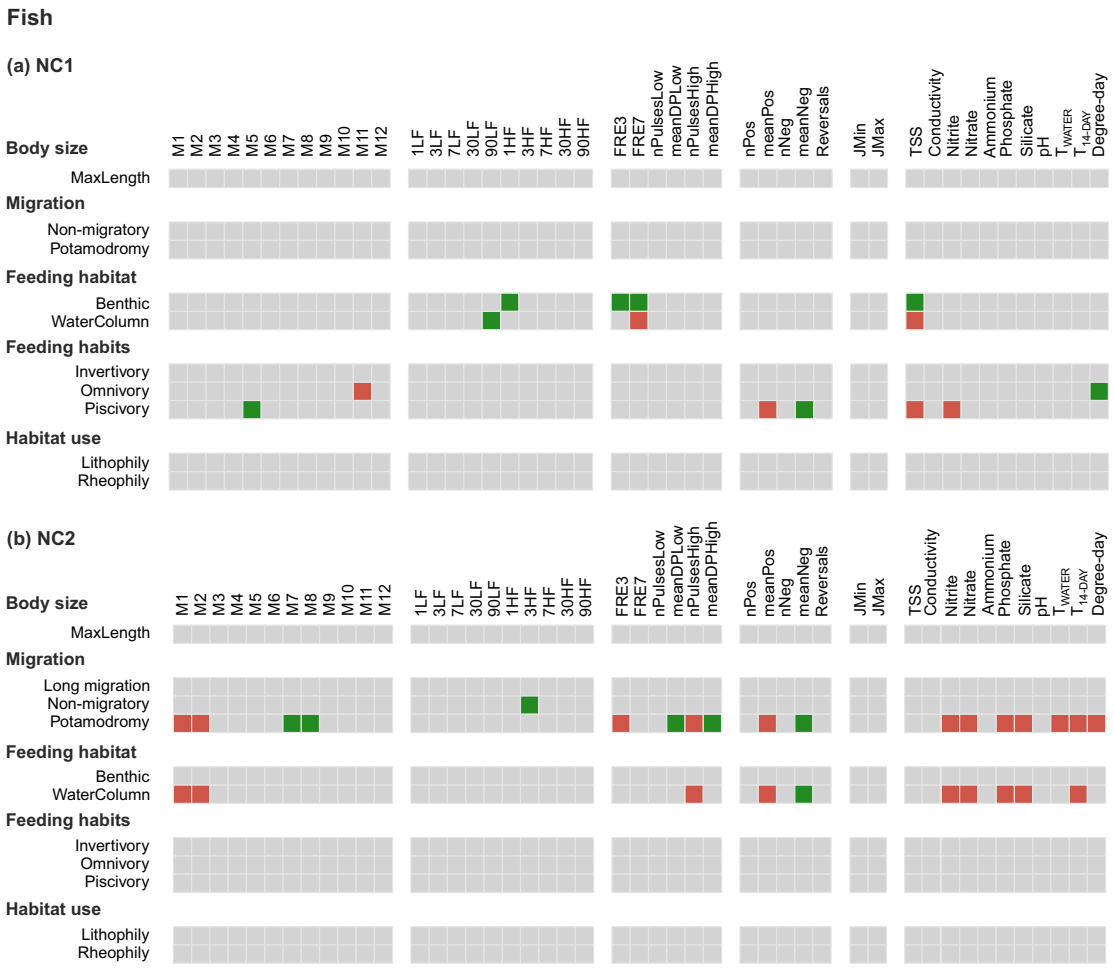


Figure S5.7 Fourth-corner correlations between fish traits (rows), hydrological (see Table A.1 for codes) and local physico-chemical variables (columns), in (a) NC1 and (b) NC2 streams. Grey boxes indicate non-significant associations (p>0.05); red boxes indicate negative significant associations; green boxes indicate significant positive associations.

Table S5.1 Taxon list of diatom taxa in NC1 and NC2 streams, divided between control (C), irrigation (IR), and hydropower (HP) sites. Abundances are means ± standard deviations across samples collected in the three years (2017, 2018, and 2019).

Code	Taxon	NC1		NC2		
		C (n=12)	**IR (n=15)**	**C (n=12)**	**IR (n=9)**	**HP (n=9)**
ADPY	*Achnanthidium pyrenaicum* (Hustedt) Kobayasi	122 ± 119.1	60.6 ± 66.5	210.5 ± 74.3	140.6 ± 109.2	112.8 ± 99.4
ADMI	*Achnanthidium minutissimum* (Kützing) Czarnecki var. *minutissimum*	30.9 ± 23	98.9 ± 78.8	62.2 ± 57.7	75.2 ± 52.7	83.1 ± 68.4
CEUG	*Cocconeis euglypta* Ehrenberg	19.9 ± 33.1	26.9 ± 30.1	24.3 ± 19	8.7 ± 7.6	16.6 ± 18.4
APED	*Amphora pediculus* (Kützing) Grunow var. *pediculus*	48.3 ± 65.1	10.1 ± 19.9	6.5 ± 8.1	2.6 ± 3.6	6.4 ± 10.3
CPLI	*Cocconeis placentula* var. *lineata* (Ehrenberg) Van Heurck	8.5 ± 17.6	5.5 ± 9.2	1.1 ± 3.8	13.1 ± 22.8	46.1 ± 81.2
NDIS	*Nitzschia dissipata* subsp. *dissipata* (Kützing) Grunow var. *dissipata*	19.3 ± 24.7	23.1 ± 33.7	5.5 ± 5.4	1.8 ± 2.3	2.6 ± 2.4
GPUM	*Gomphonema pumilum* (Grunow) Reichardt Lange-Bertalot var. *pumilum*	21.6 ± 33.3	18.7 ± 26.4	3.8 ± 8	4.2 ± 7.6	4.2 ± 4.9
NFON	*Nitzschia fonticola* Grunow in Cleve et Möller var. *fonticola*	14.3 ± 14.7	0.8 ± 1.6	25.4 ± 23.9		8.7 ± 13.9
GMIN	*Gomphonema minutum* f. *minutum* (Agardh) Agardh	4.3 ± 9.1	6.7 ± 14.6	20.9 ± 17.9	1.2 ± 3	6 ± 7.1
NCT2	*Navicula cryptotenella*/*cryptotenelloides* Lange-Bertalot var. *cryptotenella*	22.3 ± 17.9	6.6 ± 7.3	3 ± 4.8	3.1 ± 7.6	6.6 ± 6.1
NTPT	*Navicula tripunctata* (O.F.Müller) Bory var. *tripunctata*	14.1 ± 16.1	14.3 ± 19.6	3.3 ± 3.3	3.1 ± 6	1.4 ± 2.7

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Table S5.1 – *Continued from previous page*

Code	Taxon	NC1		NC2		
		C (n=12)	**IR (n=15)**	**C (n=12)**	**IR (n=9)**	**HP (n=9)**
RABB	*Rhoicosphenia abbreviata* (C.Agardh) Lange-Bertalot	13.4 ± 19.5	10.5 ± 18.4	0.9 ± 1.8	0.3 ± 0.7	0.7 ± 2
ENMI	*Encyonema minutum* (Hilse in Rabh.) D.G. Mann in Round Crawford Mann var. *minutum*	1.6 ± 2.7	6.5 ± 9.7	6.4 ± 8.8	5.3 ± 4.1	6.9 ± 8.3
ADRI	*Achnanthidium rivulare* Potapova Ponader		0.2 ± 0.8		20.7 ± 36.2	11.3 ± 17
ESLE	*Encyonema silesiacum* (Bleisch in Rabh.) D.G. Mann var. *silesiacum*	2.8 ± 2.8	6.1 ± 6.7	5.2 ± 5.7	7.1 ± 7.4	3.2 ± 6.8
ADAM	*Achnanthidium atomoides* Monnier, Lange-Bertalot Ector	4.4 ± 6.7	3.7 ± 7	7.1 ± 9.7	3 ± 5.3	4.1 ± 4.6
ADSU	*Achnanthidium subatomus* (Hustedt) Lange-Bertalot var. *subatomus*	3.9 ± 9.4	8.5 ± 15.2	0.9 ± 2.6	1.6 ± 2.7	4.6 ± 6.8
MELO	*MELOSIRA* C.A. Agardh	6.3 ± 14.7	4.1 ± 5.2	2.7 ± 2.9	1.1 ± 3.3	3.6 ± 4.9
ADRU	*Achnanthidium druartii* Rimet Couté in Rimet al.	0.3 ± 0.7	5.1 ± 7.5	0.2 ± 0.6	1.6 ± 2.5	12 ± 18.3
COPL	*Cocconeis pseudolineata* (Geitler) Lange-Bertalot	3.7 ± 6.7	3.9 ± 5.4	1 ± 1.5	1.1 ± 2.1	8.9 ± 11.4
NPAL	*Nitzschia palea* (Kützing) W. Smith var. *palea*	10.5 ± 8.3	1.7 ± 2.1	1.1 ± 1.5	1.4 ± 3.6	0.7 ± 1.4
FRUM	*Fragilaria rumpens* (Kütz.) G.W.F.Carlson	2 ± 5.1	6.4 ± 19		5 ± 6	1.1 ± 1.5
ACHD	*ACHNANTHIDIUM* F.T. Kützing	2.9 ± 3.6	3.1 ± 5.6	2.6 ± 2.9	3.9 ± 4.9	2.9 ± 4.3
CPED	*Cocconeis pediculus* Ehrenberg	1 ± 1.9	4.1 ± 5.6	2.3 ± 3.5	1.1 ± 1.7	5.4 ± 5.4
GPAR	*Gomphonema parvulum* var. *parvulum* f. *parvulum* Kützing	0.3 ± 0.8	6 ± 16.5		1.9 ± 3.3	4.9 ± 7.4
GRHB	*Gomphonema rhombicum* M. Schmidt	0.3 ± 0.9	0.3 ± 1		15.9 ± 31.4	0.6 ± 1.7

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Table S5.1 – *Continued from previous page*

Code	Taxon	NC1		NC2		
		C (n=12)	**IR (n=15)**	**C (n=12)**	**IR (n=9)**	**HP (n=9)**
DTEN	*Denticula tenuis* Kützing var. *tenuis*	0.8 ± 1.4	2 ± 2.6	1.3 ± 2.9	1.1 ± 1.8	9.1 ± 9.1
DVUL	*Diatoma vulgaris* Bory var. *vulgaris*	2.2 ± 3.6	1.7 ± 2.4	1.3 ± 2	5.3 ± 12.1	1.2 ± 2
CATO	*Cyclotella atomus* Hustedt var. *atomus*	0.1 ± 0.3	5.9 ± 14.5		2.9 ± 5.9	1.1 ± 3.3
FRAG	*FRAGILARIA* H.C. Lyngbye		1.8 ± 2.8	1 ± 2.9	3.7 ± 4.2	3.9 ± 3.4
GOMP	*GOMPHONEMA* C.G. Ehrenberg	1.9 ± 2.6	1.5 ± 2.8	1.1 ± 2.6	0.9 ± 1.5	1.2 ± 2.5
AULA	*AULACOSEIRA* G.H.K. Thwaites		1.9 ± 3.3		7.9 ± 21.9	0.4 ± 1
NCPR	*Navicula capitatoradiata* Germain	0.8 ± 1.3	2.4 ± 3.1	1 ± 1.4	0.2 ± 0.7	1.9 ± 3.9
NRCH	*Navicula reichardtiana* Lange-Bertalot var. *reichardtiana*	2.6 ± 3.8	1.9 ± 2.9	1.6 ± 3.3		1 ± 1.6
AUGR	*Aulacoseira granulata* (Ehrenberg) Simonsen var. *granulata*		2.6 ± 5.5		5 ± 7.9	
SNEO	*Stephanodiscus neoastraea* Håkansson et Hickel		5.3 ± 18.6			
CPLA	*Cocconeis placentula* Ehrenberg var. *placentula*	2.3 ± 6.6	1.2 ± 2.6	0.5 ± 1.7	0.8 ± 1.6	1.9 ± 4
FSBH	*Fallacia subhamulata* (Grunow in V. Heurck) D.G. Mann	5.4 ± 8.6			0.8 ± 2.3	0.2 ± 0.7
EOMI	*Eolimna minima* (Grunow) Lange-Bertalot	0.5 ± 0.9	0.7 ± 1.8	0.8 ± 2.6	0.2 ± 0.7	2.3 ± 3.2
RSIN	*Reimeria sinuata* (Gregory) Kociolek Stoermer	0.9 ± 1.7	1.7 ± 3.7	1.7 ± 2.7	0.2 ± 0.7	0.4 ± 0.7
TFLO	*Tabellaria flocculosa* (Roth) Kützing var. *flocculosa*		0.1 ± 0.4		6.9 ± 11.7	
AUPU	*Aulacoseira pusilla* (Meister) Tuji et Houki		3.5 ± 9.4		0.2 ± 0.7	
CYCL	*CYCLOTELLA* F.T. Kützing ex A de Brébisson		2.5 ± 6.9		0.3 ± 1	1.2 ± 2.7
NWYG	*Navicula wygaschii* Lange-Bertalot	2.8 ± 5.2		1.3 ± 3.7	0.2 ± 0.7	0.2 ± 0.7

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Table S5.1 – *Continued from previous page*

Code	Taxon	NC1		NC2		
		C (n=12)	IR (n=15)	C (n=12)	IR (n=9)	HP (n=9)
NAVI	*NAVICULA* J.B.M. Bory de St. Vincent	0.6 ± 1	0.7 ± 1.2	0.6 ± 1.2	1.8 ± 4.1	0.9 ± 1.1
NITZ	*NITZSCHIA* A.H. Hassall	1 ± 1.2	1.2 ± 1.2	0.8 ± 1.2	0.2 ± 0.7	0.7 ± 1.3
CMEN	*Cyclotella meneghiniana* Kützing var. *meneghiniana*	0.6 ± 0.9	0.4 ± 1.1	0.2 ± 0.6	1.7 ± 3.1	0.9 ± 2.3
GYAT	*Gyrosigma attenuatum* (Kützing) Rabenhorst var. *attenuatum*	1.6 ± 3.8	0.9 ± 1.9			0.3 ± 0.7
CAEX	*Cymbella excisa* Kützing var. *excisa*		0.6 ± 1.2	1.3 ± 2.7		0.7 ± 1.4
SSLE	*Staurosira leptostauron* (Ehrenberg) Kulikovsky Genkal	0.1 ± 0.3	1.3 ± 3.1	0.8 ± 1.9	0.4 ± 1.3	
ENCM	*Encyonopsis microcephala* (Grunow) Krammer var. *microcephala*		0.2 ± 0.8			3.2 ± 6.5
DPSG	*Discostella pseudostelligera* (Hustedt) Houk Klee emend. Genkal				3.3 ± 5.9	
NANT	*Navicula antonii* Lange-Bertalot	0.3 ± 1.2	1.3 ± 4.4	0.3 ± 0.8		0.2 ± 0.7
PCOS	*Pantocsekiella costei* (Druart et F. Straub) K.T. Kiss et Ács		1.7 ± 6.4		0.4 ± 1.3	
ECPM	*Encyonopsis minuta* Krammer Reichardt			1.6 ± 4.9		0.9 ± 2
CYMB	*CYMBELLA* C. Agardh	0.3 ± 0.6	0.5 ± 1.2		1.4 ± 2	0.2 ± 0.7
NAAN	*Navicula angusta* Grunow				2.9 ± 4.6	
NGRE	*Navicula gregaria* Donkin var. *gregaria*	0.5 ± 1.7		1.3 ± 2.6		0.4 ± 0.9
SCON	*Staurosira construens* Ehrenberg var. *construens*	1.1 ± 3.8	0.5 ± 1.6	0.5 ± 1.7		
UULN	*Ulnaria ulna* (Nitzsch) Compère var. *ulna*	0.1 ± 0.3	0.3 ± 0.6		0.3 ± 0.7	1.8 ± 3

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Table S5.1 – *Continued from previous page*

Code	Taxon	NC1		NC2		
		C (n=12)	**IR (n=15)**	**C (n=12)**	**IR (n=9)**	**HP (n=9)**
ESUM	*Encyonopsis subminuta* Krammer Reichardt		1 ± 2.2		0.4 ± 0.9	0.2 ± 0.7
SHAN	*Stephanodiscus hantzschii* Grunow in Cleve Grunow var. *hantzschii*		1.5 ± 3.4		0.1 ± 0.3	
ACLI	*Achnanthidium lineare* W.Smith	0.1 ± 0.3	0.2 ± 0.6	0.1 ± 0.3	1.8 ± 2.6	0.1 ± 0.3
LCMT	*Lindavia comta* (Ehrenberg) Nakov, Guillory, Julius, Theriot Alverson		1.5 ± 4.6			
STRS	*STAUROSIRA* (C.G. Ehrenberg) D.M. Williams F.E. Round	1.5 ± 4.3			0.4 ± 1.3	
GOPP	*Gomphosphenia* sp.	0.7 ± 1.8	0.5 ± 2.1	0.4 ± 1.4		
NZLT	*Nitzschia linearis* var. *tenuis* (W.Smith) Grunow in Cleve Grunow	0.7 ± 1.1	0.9 ± 1.5			
PLFR	*Planothidium frequentissimum* Lange-Bertalot var. *frequentissimum*	0.2 ± 0.6	0.5 ± 0.8			0.7 ± 1.1
FCAD	*Fragilaria candidagilae* Almeida, C. Delgado, Novais S. Blanco		0.2 ± 0.6		1.8 ± 2.3	
FVAU	*Fragilaria vaucheriae* (Kützing) Petersen var. *vaucheriae*		1.2 ± 2.7			0.1 ± 0.3
RUNI	*Reimeria uniseriata* Sala Guerrero Ferrario	0.2 ± 0.6	1 ± 2.4			0.2 ± 0.7
CLBE	*Cymbella lange-bertalotii* Krammer		0.3 ± 1	0.1 ± 0.3	1.4 ± 4.3	
DGEM	*Didymosphenia geminata* (Lyngbye) W.M.Schmidt var. *geminata*	0.5 ± 1	0.3 ± 0.7		0.8 ± 2	0.1 ± 0.3

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Table S5.1 – *Continued from previous page*

Code	Taxon	NC1		NC2		
		C (n=12)	**IR (n=15)**	**C (n=12)**	**IR (n=9)**	**HP (n=9)**
ELEI	*Encyonema leibleinii* (C.Agardh) Silva, Ludwig Menezes	0.5 ± 1.7	0.8 ± 1.3			
NCRY	*Navicula cryptocephala* Kützing var. *cryptocephala*	0.3 ± 1.2	0.7 ± 1.4	0.1 ± 0.3	0.1 ± 0.3	0.2 ± 0.7
NSOC	*Nitzschia sociabilis* Hustedt	1.5 ± 3.2				
POCL	*Pantocsekiella ocellata* (Pantocsek) K.T. Kiss et Ács		0.5 ± 1.1		1.1 ± 1.7	
AAMB	*Aulacoseira ambigua* (Grunow) Simonsen		1.1 ± 2.6			
DEHR	*Diatoma ehrenbergii* Kützing			0.4 ± 1.2	0.2 ± 0.7	1 ± 3
EUNO	*EUNOTIA* C.G. Ehrenberg		0.2 ± 0.6		1.3 ± 3.3	
NPAD	*Nitzschia palea* (Kützing) W. Smith var. *debilis* (Kützing) Grunow in Cleve Grunow					
SRBU	*Staurosira robusta* (Fusey) Lange-Bertalot		1 ± 3.9			
NMEN	*Navicula menisculus* Schumann var. *menisculus*	0.5 ± 1		0.5 ± 1.7		
APEL	*Amphipleura pellucida* Kützing		0.9 ± 2.4			
FPAV	*Fragilaria parva* Tuiji Williams	0.9 ± 2.2	0.1 ± 0.5			
LRAD	*Lindavia radiosa* (Grunow) De Toni Forti var. *radiosa*		0.9 ± 1.5			
ADPT	*Achnanthidium pyrenaicum* f. *anormale*	0.2 ± 0.6	0.1 ± 0.5	0.5 ± 0.9		0.2 ± 0.7
CBAC	*Caloneis bacillum* (Grunow) Cleve	0.6 ± 1.5	0.2 ± 0.8			
ENLB	*Encyonema lange-bertalotii* Krammer var. *lange-bertaloti*		0.2 ± 0.6		1 ± 2.3	
NCIN	*Navicula cincta* (Ehr.) Ralfs in Pritchard var. *cincta*		0.8 ± 2.1			
NNOT	*Navicula notha* Wallace				1.3 ± 2.6	

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Table S5.1 – *Continued from previous page*

Code	Taxon	NC1		NC2		
		C (n=12)	**IR (n=15)**	**C (n=12)**	**IR (n=9)**	**HP (n=9)**
NVEN	*Navicula veneta* Kützing	0.5 ± 1.7	0.1 ± 0.3	0.2 ± 0.6		0.3 ± 1
NINC	*Nitzschia inconspicua* Grunow	0.6 ± 1.1				0.3 ± 0.7
	Taxa richness	27.6 ± 9.5	31.3 ± 9.1	20.3 ± 5.2	26.3 ± 7.5	26.6 ± 6.2
	Shannon-Wiener diversity index	2.1 ± 0.6	2.3 ± 0.4	1.7 ± 0.3	2 ± 0.5	2 ± 0.3
	IPS score	16 ± 1.8	16.6 ± 1.5	17.9 ± 0.6	18.3 ± 0.9	17.1 ± 1.2

Table S5.2 Taxon list of macroinvertebrates in NC1 and NC2 streams, divided between control (C), irrigation (IR), and hydropower (HP) sites. Abundances are means ± standard deviations across samples collected in the three years (2017, 2018, and 2019).

Taxon	NC1		NC2		
	C (n=12)	**IR (n=15)**	**C (n=12)**	**IR (n=9)**	**HP (n=9)**
Orthocladiinae	349.9 ± 450	899.3 ± 923	229.8 ± 164.2	979 ± 1172.5	673 ± 522.2
Simulium	147 ± 235.8	1673.1 ± 6064.5	309.4 ± 379.4	17.7 ± 25.5	192.9 ± 189
Hydropsyche	245.9 ± 316.5	149.1 ± 226.6	173.7 ± 176.8	68.6 ± 93.2	1159.3 ± 2109
Baetis	77.7 ± 59.5	371.9 ± 411.1	254.5 ± 222.2	230.2 ± 156.3	517.7 ± 745.4
Echinogammarus	1189 ± 1511	59 ± 116.1	3 ± 4.2	1 ± 2	67.6 ± 61.8
Oligochaeta	144.2 ± 236.7	200 ± 176.8	100.2 ± 114	304.4 ± 329	146 ± 178
Elmis	199.5 ± 164.1	109.2 ± 353.8	158.5 ± 110.5	72.4 ± 113.3	330.7 ± 267.3

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Table S5.2 – *Continued from previous page*

Taxon	NC1		NC2		
	C (n=12)	**IR (n=15)**	**C (n=12)**	**IR (n=9)**	**HP (n=9)**
Chironomini	62.9 ± 105.9	43.5 ± 80.6	106.1 ± 115.8	52.1 ± 69.2	182.1 ± 232
Hydracarina	102.5 ± 141.3	59.7 ± 90.6	79.3 ± 42.2	41.6 ± 45.7	137.6 ± 99.7
Leuctra	52.3 ± 67.2	35 ± 48.7	157.6 ± 135.4	134 ± 174	25.4 ± 40.5
Tanytarsini	40 ± 40.1	50.8 ± 58.6	90.4 ± 218.5	125.3 ± 180.5	88.1 ± 80.7
Epeorus	73.6 ± 86	35.7 ± 74.8	121.3 ± 104	13.9 ± 24.3	75.7 ± 81.1
Ecdyonurus	122.9 ± 208.9	57.9 ± 66.8	33.9 ± 40.3	22.3 ± 27.7	3.2 ± 6.1
Ephemerella ignita	44.6 ± 70.7	22.5 ± 51.2	29.6 ± 44.7	98.4 ± 112.5	84.7 ± 120.9
Potamopyrgus antipodarum	14.3 ± 15.6	6.5 ± 19	59.3 ± 125.7	0.2 ± 0.7	203.9 ± 142.4
Ancylus fluviatilis	43.6 ± 54.5	36.9 ± 80.1	22.3 ± 18.8	26.3 ± 33.5	114.1 ± 110.5
Caenis luctuosa	3.8 ± 6.9	6.3 ± 9.9	0.8 ± 2.9		273 ± 410
Rhithrogena	83.7 ± 85.6	12.7 ± 23.3	101 ± 122.6	10.3 ± 16.6	0.6 ± 1.7
Limnephilidae	44.3 ± 75.1	7.9 ± 23.2	116.7 ± 239.7	39.6 ± 59.9	0.1 ± 0.3
Esolus	111.8 ± 90.5	2.4 ± 5.1	42 ± 66.7	4.4 ± 8.7	52.6 ± 136.6
Micrasema longulum	16.2 ± 38.1	27.9 ± 49	31.1 ± 79.6	32.3 ± 53.7	101 ± 115.1
Protonemoura	11 ± 22.4	59.3 ± 70.2	8.3 ± 23	46.9 ± 82.5	2.8 ± 5.7
Sericostoma	50.7 ± 80.4	1.1 ± 2.4	41.3 ± 49.4	16.8 ± 22.8	
Tanypodinae	14.4 ± 22.5	26.7 ± 38.1	12.8 ± 12.5	8.2 ± 11.8	43.2 ± 70.2
Nemoura	79.7 ± 184.9	4.4 ± 11.8	2.6 ± 7.3	4.9 ± 14.7	5.3 ± 16
Limnius	33.3 ± 28.7	5.2 ± 14.6	19 ± 14.1	8.3 ± 13.2	21.9 ± 44.4
Rhyacophila	9.8 ± 10.6	10.7 ± 14	21.2 ± 18.8	9.1 ± 12.6	34.7 ± 20

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Table S5.2 – *Continued from previous page*

Taxon	NC1		NC2		
	C (n=12)	**IR (n=15)**	**C (n=12)**	**IR (n=9)**	**HP (n=9)**
Glossosoma privatum	0.1 ± 0.3		62.8 ± 123.5	2.7 ± 5	0.4 ± 1.3
Dinocras cephalotes	3.9 ± 9.4	14 ± 28.8	10.8 ± 16.5	43.1 ± 79.5	
Ithytrichia	63.5 ± 146.5				
Lepidostoma hirtum	15.6 ± 23.4		30.8 ± 51.1	6.2 ± 16.5	16.3 ± 26.1
Habroleptoides	15 ± 26.1	36.4 ± 70.1	0.6 ± 1.4	1.1 ± 1.8	
Radix		3.3 ± 8.9	21.3 ± 66.5	30.8 ± 63.9	13.1 ± 19
Atherix	16.6 ± 22	0.1 ± 0.3	26.9 ± 26.3	3.7 ± 6	7.2 ± 14
Theodoxus fluviatilis			28.7 ± 85.7		26.6 ± 48.3
Chimarra marginata	0.2 ± 0.6		0.1 ± 0.3		61.1 ± 93.4
Pisidium	0.1 ± 0.3	27.2 ± 34.7	2.5 ± 4	4.1 ± 7	8.3 ± 23.9
Paraleptophlebia		26.7 ± 56.2	1.3 ± 4.6	14.6 ± 40.8	
Perla	24.5 ± 31.7	2.3 ± 5	6.8 ± 12	13.1 ± 25.4	0.4 ± 1.3
Micrasema moestum	6.5 ± 20.1	0.3 ± 1.3	16.5 ± 27.3	1.9 ± 5.7	24.9 ± 36.3
Physa				0.1 ± 0.3	52 ± 110.7
Oulimnius			22.4 ± 41.9		18.8 ± 33.4
Psychomyia ctenophora	4.8 ± 8	2.5 ± 6	27.9 ± 48.2		0.1 ± 0.3
Synagapetus	28.3 ± 66.1		7.3 ± 25.4		
Clinocerinae	9.7 ± 15.9	10.9 ± 13.8	2 ± 4	8.8 ± 13.3	1.8 ± 4.1
Hydraena	20.6 ± 34.1	0.1 ± 0.3	10.8 ± 14.6	0.7 ± 1.4	1.6 ± 4.7
Larcasia partita	0.1 ± 0.3		32.4 ± 77.5	0.6 ± 1.1	

Continued on next page

Table S5.2 – *Continued from previous page*

Taxon	NC1		NC2		
	C (n=12)	**IR (n=15)**	**C (n=12)**	**IR (n=9)**	**HP (n=9)**
Polycentropus flavomaculatus	4.2 ± 6.5	9.2 ± 21.2	2.6 ± 4.6	3.3 ± 8.6	15.1 ± 27.1
Hemerodromiinae	3.7 ± 6.2	5.3 ± 8.3	5.7 ± 11.5	3.7 ± 6.5	12.9 ± 31.6
Dupophilus	16.2 ± 22.3		10.1 ± 14.3		2.8 ± 7.3
Psychomyia pusilla	13.2 ± 27.7	1.1 ± 3.9	3.3 ± 9.3		10 ± 29.3
Ostracoda	0.7 ± 2.3	8.5 ± 16.3	4 ± 9.9	11.6 ± 19.2	1.8 ± 5.3
Copepoda	3.3 ± 8.2	0.6 ± 1.2	2.7 ± 9.2	8.4 ± 23.9	12.8 ± 37.2
Amphinemura			3 ± 8.2	25.4 ± 61.9	
Taxa richness	26.7 ± 6.5	23 ± 4.5	30.2 ± 3.5	26.1 ± 3.8	27.7 ± 3.2
Non-insects (0-1)	0.4 ± 0.3	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Diptera (0-1)	0.2 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.1
EPT (0-1)	0.3 ± 0.2	0.4 ± 0.2	0.5 ± 0.1	0.4 ± 0.2	0.4 ± 0.1
OCH (0-1)	0.1 ± 0.1		0.1 ± 0		0.1 ± 0.1
Shannon-Wiener index	2.1 ± 0.8	2.1 ± 0.5	2.7 ± 0.2	2.3 ± 0.6	2.6 ± 0.2
LIFE score	8 ± 0.4	7.9 ± 0.2	8 ± 0.2	7.9 ± 0.4	7.7 ± 0.4
IASPT score	5.9 ± 0.4	5.7 ± 0.4	6.1 ± 0.3	6.1 ± 0.3	5.7 ± 0.3

Table S5.3 List of fish species in NC1 and NC2 streams, divided between control (C), irrigation (IR), and hydropower (HP) sites. Abundances are means ± standard deviations across samples collected in the three years (2017, 2018, and 2019).

Taxon	**NC1**		**NC2**		
	C (n=12)	**IR (n=15)**	**C (n=12)**	**IR (n=9)**	**HP (n=9)**
Achondrostoma arcasii	0.4 ± 1.4	0.2 ± 0.8			
Anguilla anguilla			13.8 ± 20.5		4.8 ± 5.3
Barbatula quignardi	11.6 ± 22.7				
Cobitis calderoni	0.2 ± 0.6				
Parachondrostoma miegii	1.8 ± 6.4	0.1 ± 0.3		0.1 ± 0.4	
Phoxinus bigerri	31.1 ± 51.9	33 ± 44.9	31.1 ± 45.1	1.3 ± 3.5	196 ± 227
Pseudochondrostoma duriense		0.2 ± 0.6			
Salmo salar			53.3 ± 72		34.1 ± 37.2
Squalius carolitertii					8.3 ± 16.7
Salmo trutta 0+	43.9 ± 40.4	24 ± 28.1	35.4 ± 22.5	7.9 ± 4.2	4.8 ± 5.8
Salmo trutta 1+	10.3 ± 12.9	18.4 ± 25.3	18 ± 21.9	15.3 ± 9.5	0.8 ± 1.6
Salmo trutta 2+	9.3 ± 8.1	7 ± 9.6	5.3 ± 4.2	7.9 ± 9.2	6 ± 8.2
Taxa richness	2.7 ± 1.2	2.2 ± 1.3	2.5 ± 1.6	1.3 ± 0.5	3.6 ± 0.5
Shannon-Wiener diversity index	1 ± 0.3	0.9 ± 0.2	1 ± 0.3	1 ± 0.1	0.7 ± 0.3
EFI+ score	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0	0.8 ± 0	1 ± 0

Chapter 6

General conclusions and future research

The primary objective of this thesis was to assess the spatial variability patterns of biodiversity in Spanish rivers and how dams and reservoirs modify these patterns. These objectives were achieved from different perspectives (e.g. at the catchment and national spatial scales, in natural and altered regimes, and in different dam operations). Furthermore, the analysis of multiple biotic groups representing different trophic levels played a key role in this research, providing useful insights into how specific communities could be impaired by dams and, thus, which could be the following directions to ameliorate those impacts.

6.1 Influence of environmental and spatial factors on the biological communities' distribution

In Chapter 2, the spatial patterns of diatom, macroinvertebrate, and fish communities showed to be marked by an upstream-downstream gradient. However, significant ecological responses to dynamic environmental variables (i.e. hydrology, climate, land use and land cover) were also identified, suggesting that global change-related processes, such as climate change and hydrological alteration, might strongly influence the analysed riverine biological communities and cause important community shifts. Specifically, warmer climates, the development of agricultural land uses, more stable flow regimes, and calcareous geology favoured more tolerant

traits (e.g. motile diatoms, small-sized macroinvertebrates, and non-migratory fish, see Figure 6.1a). Moreover, diatom traits were more responsive to land use and land cover variables, macroinvertebrate traits to geology and LULC, while fish to climate, LULC, and hydrology.

When investigating the role of spatial (i.e. dispersal-related) and environmental factors (i.e. niche) in structuring the biological communities in Chapter 3, both factors showed a significant influence in controlling the variability patterns of diatoms and fish. In fact, spatially structured environmental factors had the highest contribution to the spatial variability of the analysed biological communities, followed by the spatial distances, and pure environmental variables. Fish communities showed a stronger spatial dependency in relation to diatoms (Figure 6.1b), which could be linked to their weaker dispersal abilities up- and downstream or across river networks and their larger body sizes. Traditional biomonitoring and conservation programmes typically ignore these aspects, assuming that community patterns are structured only on species preferences along environmental gradients. However, the biological evaluation can be impaired as new colonisers (e.g. fish or other poor disperses) may be unable to reach these sites with favourable environmental conditions, indicating, therefore, a potentially impacted site. We emphasise the need to incorporate spatial distance criteria in the monitoring design of impacts or even on the establishment of reference conditions, along with the role of environmental variables, otherwise, efforts on biological monitoring and assessment might be hampered. Moreover, the dispersal capacities of the different biological communities used as bioindicators should be considered. For example, habitat connectivity might be much better monitored with fish communities than with diatoms, while nutrient or habitat impairment might be better tracked with the second.

At the national scale, catchment-scale environmental variables (e.g. climate, land use and land cover, hydrology, topography, geology) showed a higher contribution to fish communities' variability in comparison to macroinvertebrates and diatoms in near-pristine streams. These findings indicated that fish community patterns might be strongly influenced by broad-scale environmental factors, which was confirmed in Chapters 2 and 3. In turn, these catchment-scale environmental variables had lower predictive capacity for diatom communities, suggesting they might respond to other local factors (e.g. water quality and physico-chemical characteristics, riparian conditions) not included in the analyses. Thus, we highlight that the selection of bioindicators, the scale of the study, and the scale of environmental factors might affect the riverine assessment outcomes.

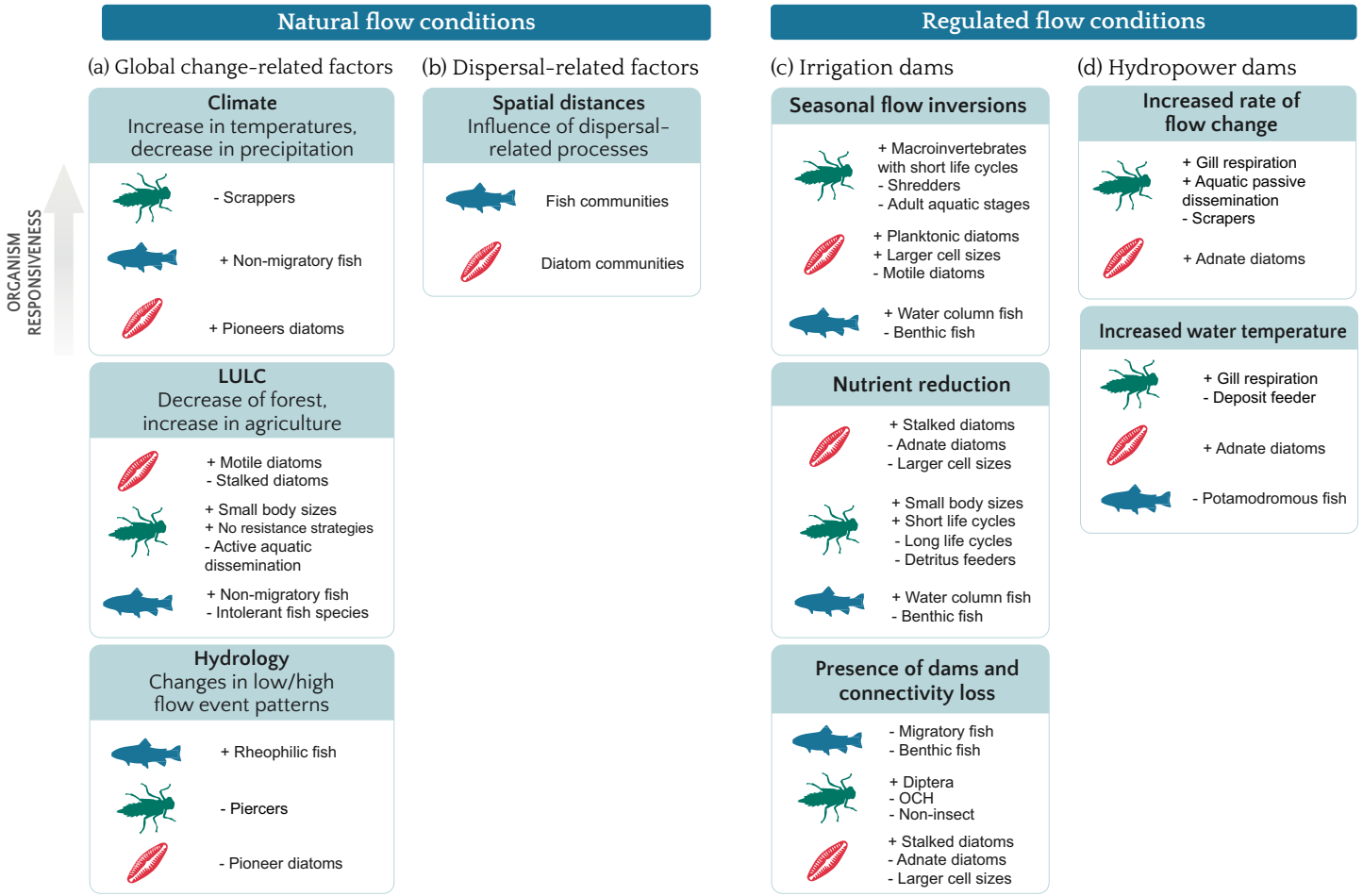


Figure 6.1 Summary of the significant ecological responses of diatom (in red), macroinvertebrate (in green), and fish (in blue) communities. Responses to the (a) main global change-related variables identified in Chapter 2 and (b) dispersal-related factors (Chapter 3). Responses to the main effects of (c) irrigation and (d) hydropower dams (Chapter 5). The order of biotic groups represents their responsiveness to the stressors in each case; signs refer to an increase (+) or decrease (-) in the trait or group.

6.2 The relevance of altered hydrological and physico-chemical conditions

The importance of flow regime components was highlighted in Chapters 4 and 5. Changes in the magnitude of monthly flows (winter and summer flows, spring flows in mountain streams) resulting in the inversion of seasonal flow patterns and decreases in the flow peak amplitude were the main hydrological alterations identified for irrigation dams (see a summary of the ecological effects in Figure 6.1c). Hydropower dams presented a high number of flow pulses with short duration, highlighting the stronger day-to-day flow variation in comparison to natural flow conditions (Figure 6.1d). Regarding physico-chemical variables, irrigation dams considerably reduced the nutrient and TSS concentrations downstream, while hydropower dams presented significantly higher water temperatures in comparison to controls located at similar altitudes.

The physico-chemical parameters also played a key role in describing assemblages' variability and in the response to dam impacts, as shown in Chapter 5. As mentioned above, these measurements were not available at the national level in Chapters 2 and 3, possibly decreasing the communities' distribution explained variation and affecting especially diatom communities, i.e. organisms more dependent on the local characteristics (niche). Thus, we emphasise the need to incorporate physico-chemical and hydrological variables when assessing the ecological patterns of riverine communities.

The 35 hydrological indices obtained from the daily discharge data showed to be valuable tools to fully describe the flow regime variation and the hydrological alteration caused by dams. Together with physico-chemical parameters, the hydrological indices allowed to establish key relationships with biological communities and their traits, as illustrated in Figure 6.2. These results reinforce the need to incorporate hydrological indices (or meaningful hydrological metrics) in conservation programmes, as well as set ecological survey sites paired with streamflow gauges.

The dam's characteristics, such as operational rules and regulation capacity, showed to be crucial factors when determining the ecological responses to dams in Chapters 4 and 5. These findings suggest that effective mitigation strategies and environmental flow guidelines should be developed taking into account specific dam features.

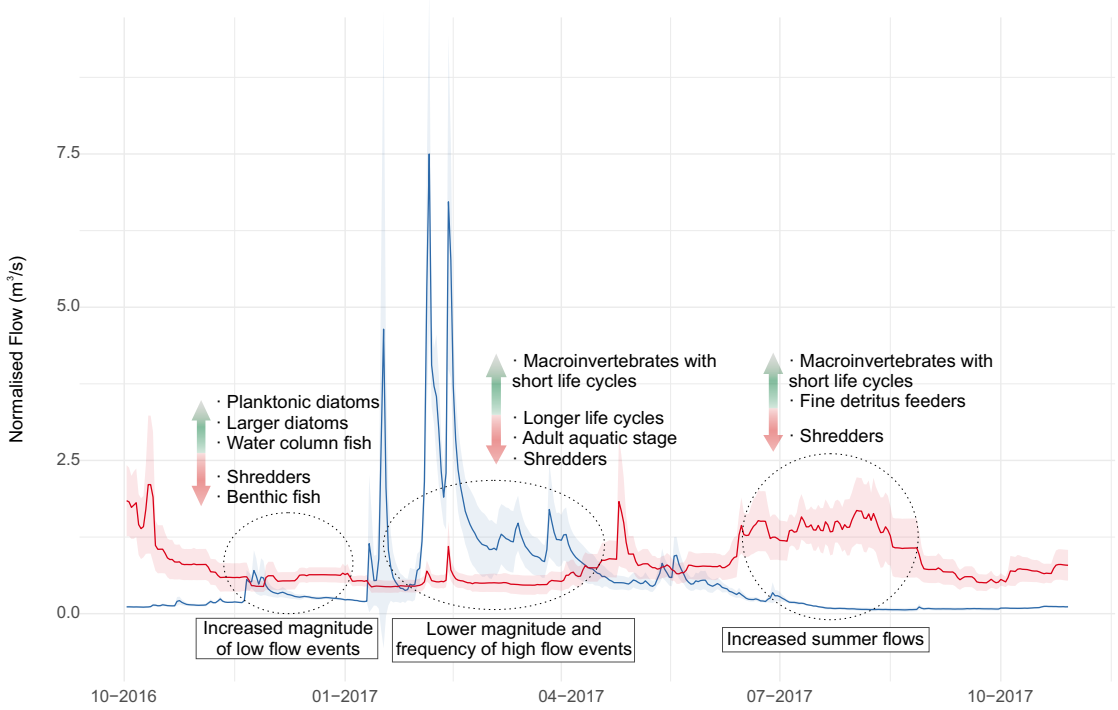


Figure 6.2 Flow regime alteration patterns caused by irrigation dams (hydrograph in red) in comparison to unregulated conditions (in blue) and the biological community shifts associated to them (Chapter 5).

6.3 Contributions to biomonitoring and conservation programmes

The findings of this thesis raised points that could contribute to the improvement of current biomonitoring and conservation strategies. The multiple biotic groups explored in this work were fundamental for gaining a deeper understanding on how specific environmental factors impaired by dams can influence three types of riverine communities. Diatoms, macroinvertebrates, and fish, i.e. organisms commonly used as biological indicators, showed variable responses to different stressors, environmental and spatial predictors, and study scales. These findings provide insights on the suitable bioindicators when monitoring different stressors and the expected community shifts, which may contribute to the development of flow-ecology relationships and more efficient biomonitoring programmes.

The use of traits throughout the chapters emphasised their advantages over taxonomy. Most of the community shifts identified in Figures 6.1 and 6.2, for example, can be associated with organisms' sensitivity to stressors or environmental gradients (e.g. more resilient traits, such as the short life cycles and small body sizes, dominate downstream of dams). The trait-based responses allowed to establish a mechanistic

understanding of how communities are structured across environmental gradients, the effects of dams, or other stressors, facilitating the prediction of ecological responses to human impacts. Moreover, the species traits allowed unifying the species pool across ecoregions, a point especially important for fish as they may present basin-specific assemblages across the country.

The use of natural hydrological classes in Chapter 5 proved to be successful in comparison to other chapters as it reduced the environmental and hydrological noise, allowing to focus on the dam-related impacts. In Chapter 4, although the site selection was limited to mountain streams, the noise generated by the environmental and hydrological variability hampered the interpretation of the dam-related effects on biological communities. Furthermore, the hydrological classification, which is an essential step in the ELOHA environmental flow framework (Poff et al., 2010), would allow us to extend the flow-ecology relationships found in the study area in Chapter 5, as well as provide flow prescriptions to other rivers belonging to the same river classes without the necessity of extensive sampling.

In summary, the results and approaches explored in this PhD thesis can contribute to the development of more effective environmental flow recommendations and other mitigation efforts for dam impacts. This is the case of using traits and hydrological classification, strategies adopted in this study to deal with the large spatial scales and can contribute effectively to the development of regional environmental flow recommendations. Besides that, the use of diatoms, macroinvertebrates, and fish and their traits emphasised the differences among these organisms in terms of response and sensitivity to specific stressors, dispersal, and scale, which are essential when designing biomonitoring programmes and meaningful flow-ecology relationships to be applied in water management issues.

6.4 Future research and recommendations

The last decades have witnessed a growing recognition of the importance of flow regimes to biodiversity and riverine ecosystems, setting the implementation of environmental flows as an urgent task. However, as identified throughout this work, further research on several aspects of river monitoring and conservation is still needed. The points listed below highlight some of these knowledge gaps, recommendations, and new research opportunities:

- The relationships between environmental predictors (e.g. environmental gradients, hydrological indices, physico-chemical variables) and biological responses (e.g. trait-based community descriptors) identified in this study were based on correlations. Thus, experimental setups in partnership with dam operators would be essential to validate these flow-ecology relationships, which would be an essential tool to develop and implement environmental flows (e.g. within the ELOHA framework) and other dam mitigation measures.

- Exploring the interactions among the three biotic groups sampled simultaneously would be interesting to better understand the riverine ecosystem dynamics and food web, as well as to determine the cascade effects of dams or other stressors, e.g. associate changes in the diatom communities to changes in macroinvertebrates and fish in a trophic cascade. Furthermore, a more in-depth evaluation of the cross-taxon congruence among the studied biotic groups would be beneficial for biomonitoring programmes.

- Current biomonitoring methods assume that communities are solely controlled by local abiotic conditions (i.e. niche approach). The incorporation of spatial (i.e. dispersal-related) processes, connectivity, and metacommunity concepts, especially in highly dynamic ecosystems (e.g. intermittent rivers, see Cid et al., 2020), would improve the accuracy of current biomonitoring approaches to detect the effects of global change factors on rivers.

- The lack of biotic indices sensitive to the dams emphasises the need to generate biotic metrics specifically dedicated to detecting hydrological alteration and other dam-related impacts. In this context, the development and calibration of biotic indices based on sensitive biological traits identified in this study would contribute to a quick assessment of the dam's impacts.

- Additional methodologies could be explored based on the relationships among flow regimes, environmental variables, and biological communities obtained in this study. For example, the construction of predictive models combining the

different taxonomic groups, temporal variation of biological surveys, and other environmental parameters (e.g. hydrological classes).

- The biological information collected for water quality assessment by national water agencies proved to be useful beyond its original purpose. Thus, the data collected for assessing the water bodies' ecological status throughout Europe (e.g. in the context of the WFD) should be catalogued in a unified database and disclosed for research purposes. Although issues with data spatial and temporal distribution and taxonomic resolution may be an obstacle to its use in research, studies on biodiversity, community patterns, and the development of preliminary flow-ecology relationships for environmental flows could be boosted with the use of large-scale riverine datasets.

- Collaborations between dam operators, water resource managers, and river researchers would be beneficial to produce long-term records of the effects of dams on riverine ecosystems and target critical knowledge gaps. For example, in the River Spöl in Switzerland, thirty experimental floods were released between 2000 and 2016, which deeply contributed to understanding the ecosystem dynamics and the long-term biota (periphyton, macroinvertebrates, and fish) responses in alpine rivers (Robinson et al., 2018). These large-scale flow experiments in cooperation with dam operators are also reported to be crucial for effectively achieving management outcomes and changing dam-operating policies (Olden et al., 2014).

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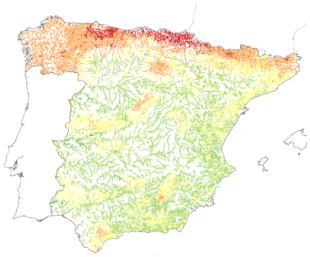
Appendix A

Hydrological indices

Table A.1 Hydrological indices used in the study. Numbers in parentheses represent the total number of hydrological indices in each group.

Group	Name	Description
Magnitude of monthly flows (12)	M1-M12	Mean monthly flows
Magnitude and duration of annual extremes (10)	1LF, 3LF, 7LF, 30LF, 90LF	Magnitude of minimum annual flow of 1-, 3-, 7-, 30-, 90-day duration
	1HF, 3HF, 7HF, 30HF, 90HF	Magnitude of maximum annual flow of 1-, 3-, 7-, 30-, 90-day duration
Frequency and duration of high and low flow pulses (6)	FRE3	Number of high flow events per year using an upper threshold of 3-time median flow within a year
	FRE7	Number of high flow events per year using an upper threshold of 7-time median flow within a year
	nPulsesHigh	Number of high pulses within a year
	meanDPHigh	Mean duration of high pulses within a year
	nPulsesLow	Number of low pulses within a year
	meanDPLow	Duration of low pulses within a year
Rate and frequency of flow change (5)	meanPos	Mean of all positive differences between days (rise rates)
	nPos	Number of days with increasing flow
	meanNeg	Mean of all negative differences between days (fall rates)
	nNeg	Number of days with decreasing flow
	Reversals	Number of hydrological reversals (flow switches from rising to falling flow, or vice-versa)
Timing of extreme events (2)	JMin	Julian day of annual minimum flow
	JMax	Julian day of annual maximum flow

Table A.2 Non-correlated synthetic indices (SI_{NAT}) and the hydrological indices with the highest contribution, see Peñas and Barquín (2019) for further details. Streams with higher SI_{NAT} scores are represented in dark red tones.

Synthetic index (SI_{NAT})	**Map**	**Description**
$SI_{NAT}1$		Negatively related intra-annual variability of daily flows (-l2) and magnitude of maximum seasonal flows (-90HF)
$SI_{NAT}2$		Positively related to the duration of high flow events (+dPHigh) but negatively associated to the annual frequency of high flow events with a threshold of 3 times the median flow (-FRE3)
$SI_{NAT}3$		Negatively related to the interannual variability of minimum flow timing (-sdJmin) and the magnitude of autumn flows (-sdM9)
$SI_{NAT}4$		Positively associated to the magnitude of spring flows (+M4) and their interannual variability (+sdM4)

Appendix B

Biological traits

B.1 Diatom communities

Table B.1 Selected functional traits for diatoms assemblages (Rimet and Bouchez, 2012)

Trait	Short name	Trait category	Examples
Ecological guild	HighProfile	High profile	*Gomphonema pumilum*
	LowProfile	Low Profile	*Cocconeis euglypta*
	Motile	Motile	*Navicula cryptotenella*
	Plantktonic	Planktonic	*Cyclotella meneghiniana*
Cell Size	Biovolume	Cell size	*Achnanthidium* (<100 µm^3)
			Cocconeis (>2000 µm^3)
Life forms	Stalk	Stalk	*Achnanthidium lineare*
	Adnate	Adnate	*Amphora pediculus*
	Pioneer	Pioneer	*Achnanthidium affine*
	Colonial	Colonial	*Encyonema minutum*
	Pedunculate	Pedunculate	*Achnanthidium pyrenaicum*

B.2 Macroinvertebrate communities

Table B.2 List of traits (biological and ecological) selected for macroinvertebrate assemblages, adapted from Tachet et al. (2010). (*) Categories with < 1% on average in all sites and, therefore, not considered in the study.

Trait	Short name	Trait category	Examples
Maximal potential size	VSmall	≤ 0.25cm*	-
	Small	0.25 - 0.5cm	*Elmis*, *Potamopyrgus antipodarum*
	SmallMed	0.5 - 1cm	Orthocladiinae, *Leuctra*
	Medium	1 - 2cm	*Hydropsyche*, *Ecdyonurus*
	MedLarge	2 - 4 cm	*Echinogammarus*, *Radix*, *Perla*
	Large	4 - 8 cm	*Lymnea*, *Haementeria costata*
	VLarge	> 8 cm*	-
Life cycle duration	Ephem	≤ 1 year	*Baetis*, Tanytarsini, *Protonemura*
	Perennial	> 1 year	*Esolus*, *Limnius*, *Perla*
Potential number of reproductive cycles	Semivoltine	< 1 per year	*Dinocras cephalotes*, *Perla*
	Voltine	= 1 per year	*Ancylus fluviatilis*, *Esolus*
	Multivoltine	> 1 per year	*Echinogammarus*, *C. luctuosa*
Aquatic stages	Egg	Egg	*Baetis*, *Hydraena*
	Larva	Larva	*Baetis*, Atherix Chironomini
	Nymph	Pupa or nymph	Tanytarsini, Chironomini
	Adult	Adult	*A. fluviatilis*, Hydraena
Reproduction	Ovoviviparity	Ovoviviparity	*P. antipodarum*, *Pisidium*
	IsolEggsFree	Isolated eggs, free	*Ecdyonurus*
	IsolEggsFixed	Isolated eggs, cemented	*Leuctra*, *Epeorus*, *Rhyacophila*

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Table B.2 – *Continued from previous page*

Trait	**Short name**	**Trait category**	**Examples**
	ClutchFixed	Egg clutches, cemented	Hydropsyche, *Baetis*, Ithytrichia
	ClutchFree	Egg clutches, free	Chironomini, Tanypodinae
	ClutchVeg	Egg clutches in vegetation*	*Calopteryx*
	ClutchTerr	Terrestrial egg clutches	*Atherix, Hydraena*, Clinocerinae
Dissemination	AquPassive	Aquatic passive	*Echinogammarus*, *Pisidium*
	AquActive	Aquatic active	*Ephemerella ignita*
	AerialPassive	Aerial passive	Chironomini, *Simulium*
	AerialActive	Aerial active	*Ithytrichia*, Clinocerinae
Resistance forms	DormantEggs	Dormant eggs, statoblasts	*Ephemerella ignita*
	Cocoons	Cocoons	Oligochaeta
	Housing	Housing against desiccation*	-
	Diapause	Diapause, dormancy	*Radix*, *Lymnaea*
	NoResistance	None	*Hydropsyche*, *Glossosoma privatum*
Locomotion	Flyer	Flyer	Gerris
	SurfaceSwimmer	Surface swimmer*	-
	WaterSwimmer	Full water swimmer	Tanypodinae, *Ithytrichia*, *Micronecta*
	Crawler	Crawler	*Leuctra*, *Epeorus*, *Radix*
	Burrower	Burrower (epibenthic)	*Pisidium*
	Interstitial	Interstitial (endobenthic)	*Atherix*, Orthocladiinae
	TempAttached	Temporary attached	*Simulium*
	AttachedPerm	Permanently attached*	-
Respiration	Tegument	Tegument	*Leuctra*, *A. fluviatilis*
	Gill	Gill	*Esolus*, *Ecdyonurus*, *Echinogammarus*
	Plastron	Plastron	*Esolus*, *Hydraena*
	Spiracle	Spiracle	*Simulium*, *G. privatum*

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Table B.2 – *Continued from previous page*

Trait	**Short name**	**Trait category**	**Examples**
	HydroVes	Hydrostatic vesicle*	-
Food	Microorgs	Microorganisms*	-
	FineDetritus	Detritus $\leq$ 1 mm	Chironomini, *C. luctuosa*
	PlantDetritus	Dead plants $\geq$ 1 mm	*Echinogammarus*, *Lepidostoma hirtum*
	Microphytes	Living microphytes	*Baetis*, *A. fluviatilis*
	Macrophytes	Living macrophytes	*Micrasema longulum*
	AnimalDetritus	Dead animals $\geq$ 1 mm	*Lymnaea*
	Microinvs	Living microinvertebrates	*Hydropsyche*, *Micronecta*
	Macroinvs	Living macroinvertebrates	*Rhyacophila*, *Atherix*
	Vertebrates	Vertebrates*	-
Feeding habits	Absorber	Absorber*	-
	DepositFeeder	Deposit feeder	Chironomini, Tanytarsini, *C. luctuosa*
	Shredder	Shredder	*Protonemura*, *Leuctra*, *Echinogammarus*
	Scraper	Scraper	*Baetis*, *Epeorus*, *Limnius*
	Filterer	Filter feeder	*Simulium, Pisidium*, *Hydropsyche*
	Piercer	Piercer	*G. privatum*, *Atherix*
	Predator	Predator	Clinocerinae, *Rhyacophila*, Tanypodinae
	Parasite	Parasite	Nematoda

B.3 Fish communities

Table B.3 List of traits selected for fish assemblages, adapted from Doadrio (2002), Schmidt-Kloiber and Hering (2015), EFI+ Consortium (2009), and Cano-Barbacil et al. (2020).

Trait	Short name	Trait category	Examples
Morphology	MaxLength	Maximum body length (cm)	*Cobitis calderoni* (9.7cm) *Anguilla anguilla* (164cm)
Migration	LongMigration	Long-distance migrants	*A. anguilla*, *Salmo salar*
	Potamodromy	Potamodromous migrants	*P. duriense*, *Luciobarbus bocagei*
	NonMigratory	Non migratory	*Phoxinus bigerri*, *Squalius carolitertii*
Overall tolerance to stressors	Tolerant	Tolerant	*Alburnus alburnus*, *Carassius auratus*
	Intolerant	Intolerant	*Alosa alosa*, *S. salar*, *Barbus haasi*
Feeding habitat	Benthic	Benthic	*A. anguilla*, *Barbatula quignardi*
	WaterColumn	Water column	*Salmo trutta*, *P. bigerri*
Feeding habits	Omnivory	Omnivorous	*P. bigerri*, *P. duriense* *Luciobarbus graellsii*
	Invertivory	Invertivorous	*Cobitis calderoni*, *S. carolitertii*
	Piscivory	Piscivorous	*S. trutta*, *S. salar* *Oncorhynchus mykiss*
Habitat use	Rheophily	Rheophilic	*P. bigerri*, *B. quignardi*
	Litophily	Lithophilic	*Parachondrostoma miegii*, *S. salar*
	Phytophilic	Phytophilic	*C. auratus*, *Tinca tinca*