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Freshwater fish in a highly regulated Mediterranean basin: biological responses to environmental stressors and to fishways implementation

Peces dulceacuícolas en una cuenca mediterránea altamente regulada: respuestas biológicas a factores de estrés ambiental y a la implementación de pasos para peces

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A mi yayo Balbino y a mi yaya Anica

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Prólogo

La presente tesis se ha dividido en diferentes apartados para facilitar su lectura y compresión, siguiendo los estándares establecidos por la Escuela Internacional de Doctorado de la Universidad de Murcia. Este documento cuenta con una introducción inicial donde se pone en contexto el problema general que da origen a este estudio, así como la finalidad y objetivos generales planteados; seguido de una descripción general del área de estudio, que será delimitada y descrita de forma concreta en cada uno de los posteriores capítulos correspondientes a los trabajos de investigación; y culmina con un apartado final donde se recogen las principales conclusiones obtenidas. Los cuatro capítulos se corresponden con los diferentes trabajos de investigación desarrollados para dar respuesta a los objetivos inicialmente planteados. Estos capítulos presentan estructura de artículo científico, incluyendo los apartados habituales (resumen, introducción, material y métodos, resultados, discusión y conclusiones), y tienen entidad autónoma para facilitar su lectura de forma independiente. Por último, todas las referencias utilizadas en este documento quedan recogidas al final del mismo.

El trabajo realizado para la consecución de esta tesis se ha realizado dentro de la línea de investigación de Biología y Conservación de Vertebrados Acuáticos y que pertenece al grupo de investigación de Zoología Básica y Aplicada a la Gestión y a la Conservación del Departamento de Zoología y Antropología Física de la Universidad de Murcia. El presente documento ha sido redactado según las indicaciones de la Escuela Internacional de Doctorado de la Universidad de Murcia (EIDUM), dentro del Programa de Doctorado de Biodiversidad y Gestión Ambiental.

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Resumen | Spanish summary

Resumen

Los ríos se encuentran en la actualidad entre los ecosistemas más impactados del planeta como consecuencia del aumento en la demanda del recurso hídrico debido al incesante crecimiento de la población humana. Las actividades vinculadas a los sistemas de agua dulce son muy diversas y, por tanto, éstos están sometidos a diferentes presiones, principalmente ligadas a su explotación para consumo humano, regadío y generación eléctrica. Las infraestructuras de regulación de caudales construidas para el aprovechamiento del recurso hídrico modifican la dinámica natural de los ríos generando, entre otros, una alteración notable en los patrones de caudal y una degradación de la calidad del agua y de los hábitats fluviales. Estas alteraciones que afectan a las especies nativas, además facilitan el establecimiento y propagación de especies exóticas invasoras, causando cambios significativos en las comunidades biológicas naturales de estos ecosistemas.

En las regiones mediterráneas de carácter semi-árido, donde el agua es un recurso escaso y los efectos del cambio climático son más acusados, la explotación intensiva del recurso hídrico hace que los impactos antrópicos sobre los ríos sean más significativos. La península ibérica es un claro ejemplo de área con ríos mediterráneos altamente alterados donde coexisten múltiples factores de estrés antrópico y sus efectos están amplificados por una fuerte presión de regulación de caudales. En España se encuentran inventariadas más de 50.000 infraestructuras de regulación, las cuales inducen complejos cambios en la dinámica natural de los sistemas fluviales, generando un incremento en la vulnerabilidad de las especies nativas. Debido a las características naturales de los ríos mediterráneos, sus comunidades de peces suelen ser pobres en especies pero con alto grado de diversificación, presentando numerosos endemismos así como especies amenazadas, lo que las hace especialmente sensibles a las alteraciones ambientales. La modificación del hábitat consecuencia de las infraestructuras de regulación no sólo incrementa el declive de las especies nativas, sino que además,

favorece a especies introducidas, conllevando que la mayoría de cuencas de la península ibérica estén actualmente dominadas por especies exóticas.

Actualmente, el estado de conservación de los ríos requiere de una especial atención debido al aumento del número y diversidad de presiones antrópicas a las que se ven sometidos, y al incremento de la magnitud de los impactos consecuencia de la acumulación e interacción entre estresores ambientales. Esto se magnifica en regiones mediterráneas donde los efectos de cambio climático son más acusados. Mitigar los impactos generados por la actividad antrópica sobre los ríos es un reto a nivel global, pero es específicamente requerido por la Comisión Europea para conseguir un buen estado ecológico de los cursos de agua en su territorio. Para ello, se considera esencial tanto una evaluación de los impactos sobre las comunidades de peces como la implementación y seguimiento de medidas de restauración.

La cuenca hidrográfica del río Segura, situada en el sureste ibérico, se localiza en una de las zonas más áridas de la península ibérica. Está caracterizada por una fuerte variabilidad típica de ríos mediterráneos, y además se encuentra sometida a una fuerte presión antrópica principalmente relacionada con la regulación de sus caudales. En consecuencia, está considerada una de las cuencas Mediterráneas más alteradas de Europa. Esto se refleja en una comunidad de peces en la cual las especies exóticas o no nativas son dominantes en la mayor parte de los tramos fluviales. Con el presente trabajo se pretende aumentar el conocimiento sobre el funcionamiento y dinámica de la comunidad de peces en sectores fluviales que están fuertemente alterados por impactos antrópicos principalmente relacionados con la regulación de caudales. Conocer la respuesta de la comunidad de peces, y también de sus especies centinela, a factores de estrés ambiental, es básico para poder implementar medidas de gestión y conservación eficaces. Además, debido a las características mencionadas (ej. alta regulación de caudales, dominancia de peces exóticos invasores), la cuenca del río Segura presenta un contexto hidrográfico óptimo para realizar este tipo de estudios a lo largo de su gradiente longitudinal.

Dentro del proyecto LIFE+ Segura Riverlink, desarrollado entre 2013 y 2017, se implementaron infraestructuras de paso para peces con la finalidad de mejorar la conectividad longitudinal en un sector del cauce principal del río Segura fuertemente fragmentado. Este nuevo escenario de gestión permitió plantear también estudios sobre la respuesta de la comunidad de peces, y de sus especies dominantes, a medidas o acciones específicas de restauración de la conectividad fluvial. Los trabajos de investigación planteados en esta tesis son de especial relevancia ya que engloban temáticas en su conjunto poco estudiadas en ríos Mediterráneos, además muy escasamente analizadas a través de estudios *in situ* en el medio natural y sobre comunidades de peces dominadas por especies no nativas.

En el contexto descrito, la finalidad de esta tesis fue estudiar la respuesta de la comunidad de peces y de una especie centinela al efecto de múltiples factores de estrés ambiental en una cuenca mediterránea altamente regulada (Cuenca del río Segura, sureste de la península ibérica). Además, se ha evaluado el uso e importancia ecológica de diferentes pasos para peces que fueron implementados como herramientas para mejorar la conectividad de un tramo fluvial altamente fragmentado. Para ello, se establecieron los siguientes objetivos específicos:

- Evaluar la respuesta de la comunidad de peces a diferentes factores de estrés ambiental a lo largo del gradiente longitudinal, identificando además rasgos biológicos relevantes que tienen un efecto significativo en la estructura y dinámica de la comunidad (capítulo 1).

- Estudiar la variabilidad en las características poblacionales de una especie centinela de ríos mediterráneos, *Luciobarbus sclateri* (Günther, 1868), ante diferentes factores de estrés ambiental presentes en el gradiente longitudinal de la cuenca (capítulo 2).

- Analizar la contribución ecológica y la efectividad de diferentes tipologías de paso para peces implementadas como herramientas de restauración de la conectividad en un sector fluvial fuertemente fragmentado (capítulos 3 y 4).

A continuación se detalla un resumen (tipo *abstract*) de cada uno de los estudios realizados en el contexto de trabajo de esta tesis para dar respuesta a los objetivos planteados. Estos estudios corresponden a los cuatro capítulos principales de la memoria.

Capítulo 1. La comunidad de peces en una cuenca mediterránea altamente regulada: respuesta funcional a factores de estrés ambiental.

Los ríos mediterráneos se caracterizan por unas condiciones ambientales extremas y unas comunidades de peces pobres en especies pero con elevado nivel de endemismo. A su vez, los ríos en Europa están expuestos a múltiples factores de estrés ambiental debido al impacto e importancia de las diversas actividades humanas. Existen estudios sobre el efecto de determinados estresores ambientales sobre algunas especies de peces, pero poco se conoce sobre la respuesta del conjunto de la comunidad de peces a la interacción de múltiples de estos factores de estrés. En este trabajo se analizó la respuesta de la diversidad funcional de la comunidad de peces a varios estresores relacionados con la degradación del hábitat y la fragmentación, la alteración del régimen de caudales y la presencia de especies invasoras en la cuenca del río Segura (sureste ibérico). Se tomaron muestras de la comunidad en 16 localidades situadas a lo largo del gradiente longitudinal de los principales ejes fluviales de la cuenca durante dos períodos (2009-2010 y 2013-2015). Posteriormente, se evaluó la respuesta de tres indicadores de diversidad funcional (especialización, originalidad y entropía), así como de la riqueza y abundancia de especies, a la interacción entre los diferentes factores de estrés tanto a escala espacial como temporal. El papel clave del régimen de caudales en la estructuración de la comunidad de peces destacó sobre otros factores de estrés relacionados con la fragmentación y cambios del hábitat físico, el deterioro del estado ecológico y la presencia de peces no nativos. En tramos fluviales con condiciones hidrológicas más estables y nivel de caudal base más elevado, se detectó un aumento de la riqueza y abundancia de especies, pero a su vez una disminución de la especialización y originalidad en la comunidad. El patrón opuesto se encontró en tramos que sufren reducciones severas del caudal base y una marcada inversión del patrón estacional de caudales altos y bajos. Según los resultados, se considera más adecuado el uso de indicadores que integran la identidad funcional (rasgos funcionales de las especies) que los indicadores clásicos basados únicamente en riqueza y abundancia de especies para evaluar los efectos de las alteraciones ambientales sobre la biodiversidad de peces. Así, el uso de indicadores de diversidad funcional puede ser más apropiado para determinar el estado de la comunidad y poder establecer medidas de conservación más eficaces. Por otro lado, las medidas de gestión basadas en el diseño de caudales que simulen patrones de régimen natural característicos de los ríos mediterráneos, se presentan como herramientas prometedoras para proporcionar condiciones ambientales que favorezcan a las especies nativas dentro de las comunidades de peces en regiones mediterráneas y, además, para limitar la proliferación de especies no nativas.

Capítulo 2. Variabilidad poblacional de una especie centinela, *Luciobarbus sclateri* (Günther, 1868), en una cuenca mediterránea altamente modificada.

La actividad humana ligada a los sistemas de agua dulce genera una amplia gama de presiones sobre el recurso hídrico. El estrés ambiental generado por estas presiones es considerado un factor determinante en la estructura y dinámica de las poblaciones de peces que habitan zonas antropizadas. En este trabajo se analizó la variabilidad de diferentes rasgos poblacionales de barbo del sur (*Luciobarbus sclateri*), especie endémica de la península ibérica, en relación con varios factores de estrés ambiental (regulación de caudales, presencia de especies invasoras, degradación del hábitat y fragmentación) a un largo del gradiente longitudinal. Durante el otoño de 2009 se muestrearon poblaciones de *L. sclateri,* así como del resto de la comunidad ictícola, en 25 localidades situadas a lo largo del gradiente longitudinal de la cuenca del río Segura, sistema fluvial situado en el sureste ibérico que presenta un fuerte impacto antrópico. Los resultados mostraron variaciones de parámetros como la edad y el crecimiento de la especie objetivo asociadas, por un lado, al efecto de la alteración del régimen de caudal y, por otro, a la abundancia de especies de peces no nativas. Sin embargo, la variabilidad de parámetros relacionados con la talla y la condición somática estuvo determinada por la interacción de varios factores de estrés ambiental con el gradiente longitudinal. Así, se encontró un incremento en las métricas relacionadas con el tamaño y la condición somática en poblaciones localizadas en tramos más bajos del gradiente, donde además también se observó el efecto del régimen de caudales, la fragmentación, las condiciones del hábitat y la abundancia de peces no nativos en la variabilidad de los rasgos biológicos de *L. sclateri*. La alta plasticidad observada en los rasgos poblacionales de esta especie centinela de sistemas mediterráneos sugiere esta plasticidad como un factor clave en la adaptabilidad de la especie para resistir en una cuenca hidrográfica mediterránea fuertemente alterada. Sin embargo, la interacción de múltiples factores de estrés juega un papel importante en la dinámica poblacional de la ictiofauna y podría inducir respuestas complejas difíciles de evaluar. Un mayor conocimiento del efecto conjunto de múltiples estresores antrópicos puede permitir un diseño más apropiado de estudios de seguimiento de peces nativos, lo cual es esencial para una mejor implementación de medidas de gestión y conservación de especies mediterráneas.

Capítulo 3. Pasos para peces en una cuenca mediterránea altamente regulada: contribución ecológica para la migración y como hábitat compensatorio en ciprínidos.

La conectividad fluvial es un factor determinante para el mantenimiento de un buen estado de las poblaciones y comunidades de peces, por lo que su recuperación es un objetivo prioritario para alcanzar un buen estado ecológico de los ríos. Ampliar el conocimiento sobre la funcionalidad de herramientas de restauración como los pasos para peces es esencial para una adecuada implementación de futuras estrategias de gestión. En el presente trabajo se evaluó la contribución ecológica de diferentes tipologías de paso para peces, construidos en una cuenca mediterránea fuertemente modificada, como herramienta para recuperar la conectividad longitudinal. Se monitorizaron cinco pasos para peces diseñados para que fuesen utilizados por especies que presentan diferentes características biológicas y requerimientos ecológicos. Se observó un uso significativamente rápido de todos los pasos, tanto de los ríos artificiales como de las escalas de artesas, por parte de la mayoría de especies presentes en la comunidad de peces. Todos los pasos fueron utilizados como corredores de migración, especialmente durante el período de movimiento por los ciprínidos potamodromos. Además, estos pasos también proporcionaron nuevos hábitats compensatorios, factor clave para juveniles y especies de pequeño tamaño, principalmente para peces reófilos en los ríos artificiales y para limnófilos en escalas de artesas. En función de los resultados, debido a las características del hábitat dentro de los pasos y a la menor variabilidad de caudal que presentan, estas infraestructuras podrían actuar como refugio en ríos fuertemente regulados con grandes fluctuaciones de caudal. Los ríos artificiales, con características más naturales, son los pasos más utilizados por especies de ciprínidos reófilos y, además, no son muy usados por grandes limnófilos no nativos, por lo que la implementación de esta tipología debería priorizarse en la planificación de medidas de restauración en ríos mediterráneos. Sin embargo, las escalas de artesas podrían ser utilizadas como trampas de control de algunas especies no nativas, y su construcción podría ser de interés para reducir el riesgo de propagación de peces exóticos invasores. La información obtenida como resultado de este trabajo sobre la funcionalidad ecológica de pasos para peces diseñados para múltiples especies, debe considerarse para conseguir mejores resultados en la implementación de medidas de restauración, lo cual es esencial para cumplir con los requisitos la Directiva Marco Europea del Agua.

Capítulo 4. Comportamiento de paso de ciprínidos potamodromos en escalas de artesas implementadas en una cuenca mediterránea semi-árida.

Las especies de peces endémicos de agua dulce de ambientes semi-áridos se encuentran entre las más amenazadas del mundo debido a la sobreexplotación del recurso hídrico y entre otros, a los problemas de fragmentación del hábitat que genera. Los pasos para peces de estanques sucesivos o escalas de artesas son utilizados en todo el mundo como herramienta para restablecer la conectividad longitudinal y mitigar los problemas de migración de peces. Esta tipología ya se ha implementado en ríos de ambientes semi-áridos, sin embargo, el comportamiento de paso de los peces a través de estas infraestructuras ha sido escasamente evaluado en regiones mediterráneas. El presente trabajo se centra en el estudio del comportamiento de dos ciprínidos potamódromos, el barbo del sur (*Luciobarbus sclateri* (Günther, 1868)) y la boga del Tajo (*Pseudochondrostoma polylepis* (Steindachner, 1864)), dentro de dos escalas de artesas de diferente tipología: de hendidura vertical y de vertederos sumergidos con orificios de fondo. Los experimentos se llevaron a cabo durante el periodo de movimiento o migración de estas especies en el río Segura (sureste de la península ibérica), utilizando un sistema de seguimiento de antenas y microchips pasivos (PIT-tag). El éxito en el ascenso fue superior al 80% para las dos especies, con un tiempo medio de tránsito inferior a 17 minutos por metro de altura en todas las pruebas, tanto por especies como por tipología de paso. Los resultados muestran que ambos tipos de escalas de artesas, si se diseñan y construyen correctamente, ofrecen alternativas interesantes para la restauración de la conectividad longitudinal y la recuperación de las vías de migración de los peces en regiones mediterráneas.

Conclusiones generales

Tras la realización de los trabajos de investigación propuestos en esta tesis con el fin de estudiar la respuesta de la comunidad de peces, también de una especie centinela, al efecto de múltiples factores de estrés ambiental, así como de evaluar el uso e importancia ecológica de diferentes tipologías de paso para peces, se extraen las siguientes conclusiones generales:

- Los factores de estrés ambiental presentes en la cuenca del río Segura, así como su magnitud e interacción, definen la composición y estructura de la comunidad de peces determinando los rasgos biológicos que permiten a las especies habitar en un ambiente condicionado principalmente por la regulación de caudales. El uso de indicadores de diversidad funcional se ha mostrado más apropiado que los indicadores clásicos de riqueza y abundancia para evaluar los efectos de impactos antrópicos sobre la comunidad de peces.

- Se confirma la alteración del régimen de caudales como factor determinante en la composición y estructura de la comunidad de peces de tramos fluviales. Así, el aumento de caudal base y de la estabilidad de caudales promedio, junto con el incremento de la fragmentación longitudinal, conllevan una mayor presencia de especies no nativas y facilita la proliferación de generalistas. El resultado es una homogeneización de la comunidad con pérdida de originalidad y especialización, a pesar de ver incrementada su riqueza específica y abundancia.

- La edad y crecimiento en las poblaciones de *Luciobarbus sclateri* mostró una relación significativa con la alteración del régimen de caudales y la abundancia de peces no nativos, sugiriendo que la magnitud de estos impactos antrópicos condiciona las respuestas biológicas esperadas para la especie en el gradiente longitudinal. Por otro lado, métricas con mayor plasticidad individual como la condición somática y la talla mostraron su variabilidad asociada al gradiente longitudinal, con mayores valores de estos parámetros en tramos bajos donde confluyen efectos relacionados con el régimen de caudales, fragmentación y abundancia de peces no nativos. A su vez, las poblaciones establecidas en tramos aguas abajo de los grandes embalses muestran una estructura poblacional afectada, con reducciones importantes de individuos de pequeña talla.

- La alta plasticidad observada en los rasgos poblacionales de *Luciobarbus sclateri* a lo largo del gradiente longitudinal sugiere que dicha plasticidad es un factor clave en la capacidad de la especie para habitar en una cuenca fuertemente alterada. No obstante, se han confirmado asociaciones relevantes entre impactos derivados de la regulación de caudales sobre dichos rasgos, proporcionando información sobre las respuestas a nivel de población de este endemismo ibérico catalogado como centinela de ríos mediterráneos.

- El presente trabajo muestra que los pasos para peces diseñados para especies con diferentes requerimientos y características biológicas (pasos multi-específicos) representan una contribución ecológica de importancia para las especies dominantes (ciprínidos) en tramos fluviales de la cuenca del río Segura. Ampliar el conocimiento sobre la funcionalidad de estas herramientas de restauración de la conectividad en cuencas mediterráneas es básico para la implementación de estrategias de gestión.

- Los pasos multi-específicos, tanto los ríos artificiales como las escalas de artesas, fueron más utilizados por ciprínidos potamodromos durante sus periodos de movimiento, indicando su funcionalidad para la migración. A su vez, estos pasos también proporcionaron nuevos hábitats compensatorios mayormente para las especies reófilas, también para limnófilas en el caso de las escalas de artesas. Las características del hábitat y del caudal circulante dentro de los pasos pueden conllevar que estas infraestructuras actúen como refugio en tramos con grandes fluctuaciones de caudal, siendo una contribución ecológica clave para los juveniles y ejemplares de pequeño tamaño.

- La implementación de ríos artificiales debería priorizarse en la planificación de medidas de restauración de la conectividad entre poblaciones de peces en ríos mediterráneos ya que, al reproducir mejor las características naturales de tramos fluviales, son los pasos más utilizados por ciprínidos reófilos y los menos usados por grandes especies exóticas de carácter limnófilo. No obstante, las escalas de artesas, tanto de hendidura vertical como de vertederos sumergidos con orificios de fondo, con un diseño y construcción correcta son alternativas muy funcionales para la restauración de la conectividad y la recuperación de vías de migración de ciprínidos potamodromos en regiones mediterráneas. Además, estas escalas de artesas podrían ser utilizadas como trampas de control de algunas especies no nativas y útiles para reducir la propagación de peces invasores.

- En suma, la magnitud e interacción de impactos a los que se ven sometidos los ríos altamente regulados del área mediterránea dificulta la interpretación de las respuestas a dichos impactos en la comunidad y poblaciones de peces. El aumento en el conocimiento de las respuestas biológicas a diferentes escalas, de tramo fluvial y gradiente longitudinal, debe permitir una mejora en la implementación de medidas exitosas de conservación y restauración.

General introduction and objectives

Since fresh water is considered the most essential natural resource, rivers are considered worldwide among the most altered ecosystems by human impacts (Meybeck, 2003; Sabater et al., 2018). Historically, a wide range of activities have been developed to meet human needs and multiple environmental stressors alter the hydrology and water quality of rivers, directly or indirectly affecting the freshwater biodiversity (Ormerod, 2003; Srinivasan et al., 2012; Vörösmarty et al., 2000).

During the last decades, human impacts related to flow management to take advantage of water resources have increased due to the intensification of water demand from a growing human population (Dudgeon et al., 2006; Grill et al., 2015). The complex interaction of different environmental stressors is expected to be intensified by their cumulative effects and the presence of new emerging threats identified in recent years (Reid et al., 2019). In particular, artificial barriers are increasing the effects of multiple human stressors by altering flow patterns, amplifying the effects of increasing amounts of pollutants discharged into freshwater, causing severe degradation of the habitat and water quality and favouring the establishment and spread of exotic species, which cause changes in biological communities (Ormerod et al., 2010; Schinegger et al., 2016).

In semi-arid Mediterranean regions where freshwater is scarce and the effects of climate change are exacerbated, the intensive exploitation of the water resource induce more significant impacts in rivers than in other climatic regions (Veldkamp et al., 2017; Vörösmarty et al., 2000). In these regions, rivers are naturally characterized by strong changes in water availability and the flow regime is considered the main driver of fluvial dynamics, so here the alteration of hydrological patterns severely affects freshwater biodiversity (Gasith and Resh, 1999; Hooke, 2006). The Iberian Peninsula is a clear example of an area with heavily altered Mediterranean river systems where multiple human-induced stressors coexist, and their effects are amplified under the pressure of strong flow management (Bonada and Resh, 2013; Sabater et al., 2018). In Spain, a large number of flow management infrastructures (>50,000 obstacles) have been built since 1960 (Brink et al., 2018; Maceda-Veiga, 2013), inducing complex changes in river dynamics that increase vulnerability and create a special risk for native species (Bunn and Arthington, 2002; Hershkovitz and Gasith, 2013; Poff and Zimmerman, 2010).

The fish species in Mediterranean rivers are adapted to the natural strong variability and harsh environmental conditions (Encina et al., 2006; Hershkovitz and Gasith, 2013). The Iberian Peninsula has a large number of complex hydrographic basins, most of them small and independent, which often implies the isolation of fish populations and a high level of species diversification. Iberian freshwater fish assemblages are usually poor in species, and show a high level of endemism and threatened species (Clavero et al., 2004; Doadrio, 2002), so they are especially sensitive to human-induced alterations (Fornaroli et al., 2020; Hermoso and Clavero, 2011). Fish are particularly affected by flow management obstacles that prevent their free movement along the river during spawning periods or in search of feeding areas (Rincón et al., 2017). In addition, changes in habitat and water quality derived from artificial barriers also affect the structure of the fish assemblage since environmental conditions act as a filter selecting optimal species to inhabit these areas according to their biological traits (Hermoso et al., 2017; Lucas and Baras, 2001; Poff, 1997). In heavily regulated rivers such as those of the Iberian basins, environmental alterations not only increase the decline of native species, but also favour the establishment and proliferation of various exotic species (Ilhéu et al., 2014; Rahel, 2007), the latter being dominant in most of the rivers of the Iberian Peninsula (Aparicio et al., 2012; Clavero et al., 2013; Ferreira et al., 2007; Fornaroli et al., 2020).

With anthropogenic impacts on the increase, threats such as the river fragmentation are increasing while climate change is exacerbated in Mediterranean regions; the enhancement of human impacts due to their interaction and cumulative effect especially affects the freshwater fauna in these threatened ecosystems (Reid et al., 2018; Vörösmarty et al., 2000). Therefore, special attention is needed regarding the conservation status of Mediterranean rivers. Mitigating the effects of human impacts is a global challenge in order to restore more natural dynamics in rivers (Pringle, 2003), but in particular to achieve a good ecological status of freshwater systems as established in the European Water Framework Directive (European Comission, 2000). A preliminary assessment of the influence of human impacts on the fish assemblage is essential to implement successful restoration measures, but equally important is the assessment of the species response to the restoration tools, which confirms its success and also highlights needs for improvement.

The Segura River basin, a small drainage basin located in the south-east of the Iberian Peninsula, is placed in an area considered among the most arid Mediterranean regions (Vidal-Abarca et al., 1992). This basin displays a strong natural variability characteristic of Mediterranean rivers and, in addition, it is affected by strong human impacts mainly related to flow regulation (Belmar et al., 2013; Bruno et al., 2014), being considered among the most highly regulated Mediterranean basins (Grindlay et al., 2011). Flow management entails a strong fragmentation impact and flow regime alteration that induces severe changes in fluvial habitats and water quality (Ilhéu et al., 2014; Schinegger et al., 2016). In this basin, multiple environmental stressors affect the fish assemblage structure, where non-natives are dominant in most of its rivers, with different families ranging from exotics to the Iberian Peninsula to those translocated from nearby basins, and with a single native species well distributed throughout the entire basin (Oliva-Paterna et al., 2019, 2014). The aim of the present study is to increase knowledge on the functioning and dynamics of both fish populations and assemblages in a Mediterranean region strongly altered by human impacts mainly related to flow management. Assessment of the response of the fish assemblage, including its sentinel species, to environmental stressors as well as to measures implemented to restore longitudinal connectivity, is essential in order to implement effective management and conservation strategies. In addition, due to the basin characteristics of the Segura River (e.g., strong flow management, non-native dominance); it offers a good opportunity to conduct this type of study along longitudinal gradients.

In order to improve the longitudinal connectivity, several fish passage infrastructures were implemented in the context of the LIFE + Segura Riverlink project, developed from 2013 to 2017. This restoration strategy enabled assessment of the response of the fish assemblage, as well as its dominant species, to the specific restoration measures implemented to mitigate the impacts of human activity. These studies, especially in the field, are rarely conducted in Mediterranean rivers and on fish assemblages dominated by non-native species.

In this context, the purpose of this thesis was to study the dynamics of both the whole fish assemblage and a native sentinel species, under the effects of human-induced stressors in a strongly regulated Mediterranean basin. In addition, this study also assessed the ecological implications of multi-species fishways as a tool to restore the longitudinal connectivity in a highly regulated system. In order to achieve this objective, the following chapters were focused on specific aims:

- Assessment of the functional response of the fish assemblage to environmental stress factors along the longitudinal gradient, using a trait-based approach that considers the role of all the species present in the fish assemblage (Chapter 1).
- Study of the population traits variability of the native *Luciobarbus sclateri* related to environmental stressors described along the longitudinal gradient (Chapter 2).
- Evaluation of the ecological contributions and effectiveness of different typologies of multi-species fishways built as a tool to restore the longitudinal connectivity in a heavily regulated river (Chapters 3 and 4).

General description of the study area

Study area and human impacts

This study was conducted in the Segura River basin, placed in the south-east of the Iberian Peninsula and considered a medium-small basin representing 3.7% of Spanish territory (Figure 1). This basin drains an area of 18.870 km^2 that mainly concerns the region of Murcia (59% of the total area) but also other three different national administrative regions: Castilla-La Mancha, Andalucía and Valencia (CHS, 2007). There are two large permanent rivers: the Segura River as the main stem with approx. 235 km in length and the Mundo River as the main tributary with a total of 119 km. This study was focused in permanent river reaches of the middle and upper part of the Segura River basin. Different number of sampling sites was involved at each chapter, all of them placed in the first 200 kms of the main stem (the Segura River), along the Mundo River and in two small upstream tributaries (Tus and Taibilla).

The Segura River basin is located in a semi-arid Mediterranean region and is characterised by scarce rainfall (300 mm/year approx.) and warm temperatures (mean annual temperature approx. 18 °C), but despite its not very large extension, this basin presents a strong climatic and altitude gradient. From headwaters in areas above 2000 m.a.s.l. to extensive plains near to the coast, the Segura River basin shows areas where rainfall exceeded 1000 mm/year and the temperature reaches values below 0ºC during winter, but also extreme aridity in places with rainfall under 250 mm and summer temperatures above 40ºC (CHS, 2007; Vidal-Abarca et al., 1987). In general, the rainfall is characterized by strong temporal variability, but normally presents large dry seasons mainly during the hot summers and rainy periods that are frequently torrential especially during autumn (Miró et al., 2018).

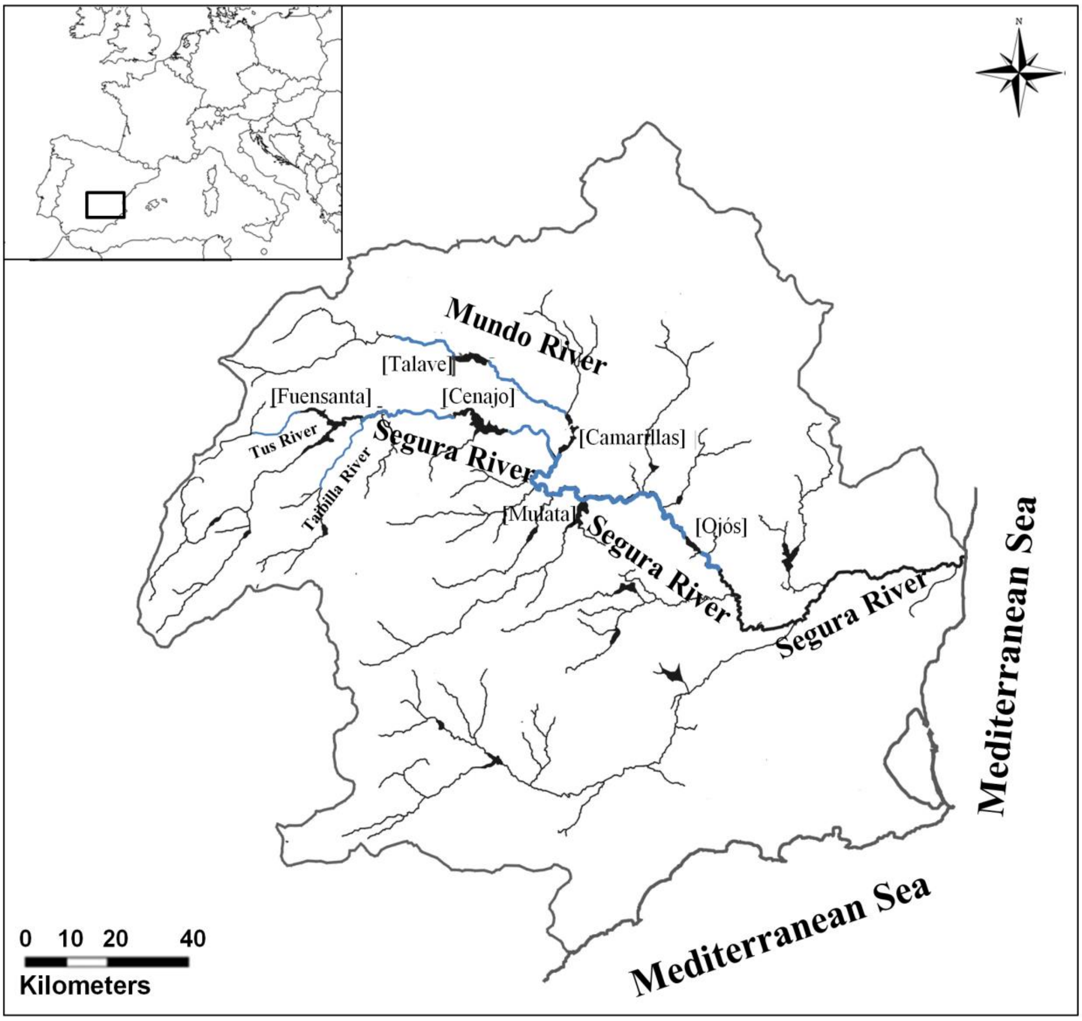

Figure 1 Study area. Location of the Segura River basin in the south-east of the Iberian Peninsula. The rivers used in this study are named and the reaches involved are highlighted in blue. Names of large reservoirs are between parentheses [].

Historically, multiple human activities which imply a strong water demand have been developed in this basin. Here, human impacts coexist with the natural variability characteristic of Mediterranean rivers and with the scarcity of the water resource, being its fluvial and riparian habitats severely altered (Belmar et al., 2011; Bruno et al., 2014). Agricultural practices are the main human pressures showing 90% of the water demand, but also hydropower generation and human supply are involved in the overexploitation of water resource (Belmar et al., 2013). Meeting human needs has entailed strong flow management: a total of 33 large dams (>10 m height and >1 hm^3 of reservoir) with approximately 1200 hm^3 of a total storage capacity, and around 170 smaller obstacles such as weirs exist along the longitudinal gradient, being considered one the most intensively regulated Mediterranean basins (Grindlay et al., 2011). Furthermore, since 1979 this basin has received an inter-basin water transfer of an average of 350

hm^3year^{-1} from the Tajo River to the Mundo River, which implies an increase of approx. 140% of its natural input (CHS, 2013; Grindlay et al., 2011). Flow management generates a strong alteration of the natural flow regime, but in addition, cause changes in habitat and water quality, which are increased by pollutants discharged into freshwater as a result of agricultural practices (CHS, 2013).

Other strong human pressure severely affecting the Segura River basin is the introduction of non-native species. There are different ways of introductions: from deliberate, such as the historical introduction of *Cyprinus carpio* in 17th century (Elvira and Almodóvar, 2001) or the recently introduced *Alburnus alburnus* (Amat-Trigo et al., 2019); to unintentional as for example *Gobio lozanoi* by means of the inter-basin water transfer (Elvira and Almodóvar, 2001). The easily establishment and spread of foreign species is mainly conditioned by the environmental conditions which are severely altered in this area, so non-native fish species tend to be dominant in the fish assemblage of the Segura River basin in the last decades (Oliva-Paterna et al., 2014).

The flow regime characterisation

The Segura River basin shows significant annual and inter-annual hydrological variability. The natural flow regime in this Mediterranean river is characterized by marked seasonal variations with severe droughts during the summer and flow peaks mainly during spring and autumn (CHS, 2007; Miró et al., 2018). But natural flow conditions are currently relegated to few reaches in head waters due to the presence of flow management infrastructures distributed throughout its longitudinal gradient. Agricultural practices require a large amount of water during the summer when the water level is naturally low in this area. So, flow regulation drastically impacts the flow pattern causing a strong inversion in seasonality with high flow levels during spring-summer and low levels in autumn-winter (Aldaya et al., 2019; Vidal-Abarca et al., 1990) (Figure 2). Furthermore, each particular dam operation could cause a different flow impact: from more constant and high level of base flow throughout the year and great water temperature (e.g., downstream La Mulata dam) to high level of contingency (similar patterns inter-years) and low daily flow variation but less monthly predictability (e.g., downstream Cenajo reservoir), giving an impact gradient along the study area more extensively described by Amat-Trigo (2018).

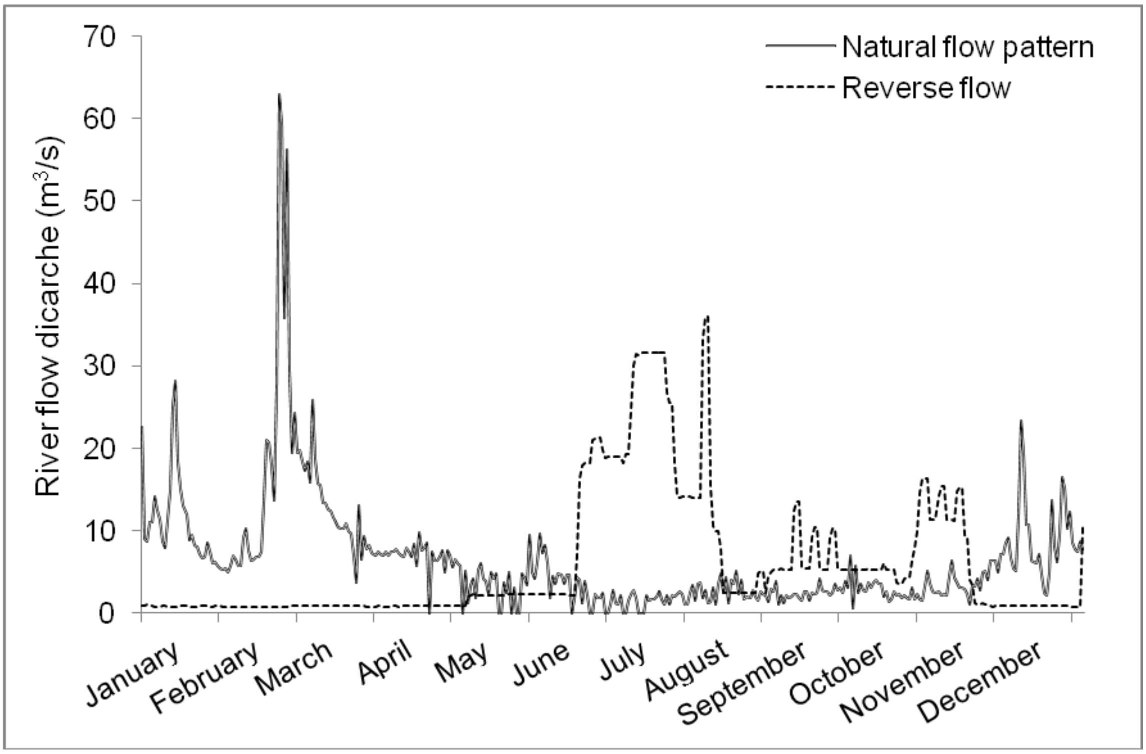

Figure 2 Example of natural flow pattern and the seasonal inversion (reverse flow) caused by flow management. Data correspond to the river flow discharge (m^3/s) during 2010 in two gauging station: natural flow pattern at the upper part of the Mundo River, and reverse flow just downstream of the Cenajo reservoir.

Extreme impacts and the natural flow pattern that occurs in the study area are represented in some chapters of this study during different periods (e.g. Figure 1.2 in chapter 1). The different flow patterns were characterised using the same the hydrological metrics but different temporal data series. Two hydrological metrics were calculated by means of river discharge data from the main gauging stations distributed along the upper and middle part of main stem of the Segura River basin (the Segura River), and the main tributary (the Mundo River) (CHS, 2007). These data were obtained from the Segura Hydrographic Confederation (CHS) online databases. Mean daily values of flow discharge (m^3 s^{-1}) were used to calculate the Mean Daily Base Flow (MDBF) as the total base flow component of the hydrograph divided by the number of recording days, and flow variability as the daily range between Q10% and Q90% discharge divided by the median value, using time series analysis (TSA) of the River Analysis Package (RAP version 3.0.7) (Marsh et al., 2003).

The fish assemblage

The fish assemblage in the Segura River basin has undergone significant changes in the last decades, being currently dominated by non-native fish species (Oliva-Paterna et al., 2014, 2019). The altered environmental conditions, mainly as consequence of flow management, has favoured the establishment and spread of multiple introduced species that are currently abundant and widely distributed throughout the entire system (Martínez-Morales et al., 2010; Oliva-Paterna et al., 2019; Torralva et al., 2005). This study was focused in the upper and middle part of the Segura River basin, so species from transitional waters such as estuaries, coastal lagoons and marshlands were excluded. Therefore, a total of 15 freshwater fish species were considered in this study that involved the upper and middle part of the basin, where non-natives (exotic to the Segura River basin) account 80% of total species richness of this poor species assemblage (Table 1).

Table 1 Freshwater fish species currently present in the study area. (N: native, E: exotic to the Segura River basin, T: translocated from Iberian basins). Common names (English and Spanish) for species according to (Leunda et al., 2009; Oliva-Paterna et al., 2019).

Family	Specie	Common name/Spanish common name	Origen
Cyprinidae	*Alburnus alburnus* (Linnaeus, 1758)	Bleak/Alburno	E
Cyprinidae	*Carassius auratus* (Linnaeus, 1758)	Goldfish/Carpín dorado	E
Cyprinidae	*Cyprinus carpio* Linnaeus, 1758	Common carp/Carpa común	E
Cobitidae	*Cobitis paludica* (de Buen, 1930)	Southern Iberian spined-loach/Colmilleja	E (T)
Esocidae	*Esox lucius* Linnaeus, 1758	Northern pike/Lucio	E
Poeciliidae	*Gambusia holbrooki* (Agassiz, 1859)	Eastern mosquitofish/Gambusia	E
Cyprinidae	*Gobio lozanoi* Doadrio & Madeira, 2004	Pyrenean gudgeon/Gobio	E (T)
Centrarchidae	*Lepomis gibbosus* (Linnaeus, 1758)	Pumpkinseed/Pez sol	E
Cyprinidae	*Luciusbarbus sclateri* Günther, 1868	Southern Iberian barbel/Barbo del sur	N
Centrarchidae	*Micropterus salmoides* (Lacépède, 1802)	Largemouth black bass/Perca americana	E
Salmonidae	*Oncorhynchus mykiss* (Walbaum, 1792)	Rainbow trout/Trucha arcoíris	E
Cyprinidae	*Pseudochondrostoma polylepis* (Steindachner, 1864)	Iberian straight-mouth nase/Boga del Tajo	E (T)
Percidae	*Sander luciusperca* (Linnaeus, 1758)	Pike perch/Lucioperca	E
Cyprinidae	*Squalius pyrenaicus* (Günther, 1868)	Southern Iberian chub/Cacho	N
Salmonidae	*Salmo trutta* Linnaeus, 1758	Brown trout/Trucha común	N

Cyprinids are dominant in the study area, with seven out of 15 species present in the fish assemblage (Oliva-Paterna et al., 2019). Four of these cyprinids are the most abundant and widely distributed species: the exotic bleak, two translocated Iberian species (Iberian straight-mouth nase and Pyrenean gudgeon), and the Southern Iberian barbel that is the most widely distributed native species, being the only native in many river reaches. Other cyprinids such as the native Southern Iberian chub (relegated to the most natural areas) and the exotic common carp (in warm and lentic areas) show locally abundant populations; whereas the goldfish only occasionally was detected in downstream reaches were it finds warm and low current waters. Species from different families are also present in the study area. The translocated Southern Iberian spined-loach and the native brown trout are only present in upper part, where river reaches present the most cold and running waters of the Segura River basin and a better water quality. The other salmonid present in the study area (the exotic rainbow trout) shows a specific distribution due to its intentional introduction. This species is usually released in sets of individuals into the river for recreational fishing, so most of its captures normally occur near the places where they are released. In the case of the medium-sized predator pumpkinseed, as well as the large piscivores species present in the study area: largemouth black bass, pike perch and northern pike; all of them are mainly present in reservoirs and medium-low river reaches with low current waters, high amount of vegetation and refuge. In the case of the small exotic mosquito fish, it is well distributed in medium and low reaches and also is present in many different agriculture-storage infrastructures (Oliva-Paterna et al., 2019, 2014).

Chapter 1

Fish assemblages in a highly regulated Mediterranean river: functional response to environmental stressors

Abstract

Mediterranean rivers are characterised by strong environmental constrains and species-poor, highly endemic fish fauna. In Europe, these systems are exposed to multiple stressors due to extensive human activities. Studies on the effects of some stressors on riverine fish are available but complex responses offish assemblages to inter- play of flow alteration with physical habitat changes and invasive species have not been evaluated up to date. This study analysed the response of functional diversity of fish assemblages to multiple stressors in the Segura River basin in the southern Spain. Fish assemblages were sampled in 16 sites in two consecutive periods (2009–2010 and 2013–2015). Subsequently, we assessed the responses of functional specialisation, originality and entropy (based on nine functional traits and abundances) as well as species richness and abundance to inter- play of flow regime alteration and ecological status, fragmentation as well as non-native species abundance across spatial and temporal scales. The governing role of flow regime in structuring fish assemblage was superimposed on physical habitat changes, water quality deterioration and fragmentation as well as the presence of non-native fish species. We found an increase of species richness and abundance but decrease of functional specialisation and originality in river reaches with high level of base flow and more stable hydrological conditions. Opposite pattern was observed in reaches with severe reduction of base flow and marked inversion in the seasonal pattern of high and low flows. We postulate that the use of tools that consider the functional identity of the species as method to assess the effects of environmental alterations on fish biodiversity could improve conservation measures for Mediterranean fish fauna. Furthermore, design flows that mimic natural flow regime patterns characteristic for Mediterranean rivers are a promising tool to provide environmental conditions that would favour native fish within the assemblage and benefit their conservation.

1.1 Introduction

Rivers are among the most altered natural systems as a result of multiple stressors (Saunders et al., 2002). Stressors such as fragmentation, pollution and flow regime alteration cause significant alterations of natural hydrology and geomorphology and affect water quality and biological assemblages of rivers (Ormerod et al., 2010; Schinegger et al., 2016). As a consequence, many river systems worldwide have experienced severe declines in their biodiversity in recent decades (Dudgeon et al., 2006; Olden et al., 2010). Furthermore, climate change exacerbates demand of water resources that augments the effects of other stressors, and both climatic and non-climatic factors often give advantages to the non-native species (Maceda-Veiga, 2013; Vörösmarty et al., 2000). Recent studies have evaluated responses of fish assemblages in river systems to individual stress factors such as fragmentation caused by dams (Liu et al., 2018), non-native fish introductions (Marr et al., 2013; Toussaint et al., 2018), flow regime alterations (Oliveira et al., 2018) or habitat degradation (Brandl et al., 2016). However, the majority of European rivers are exposed to multiple stressors, which in most cases induce complex changes in their ecological dynamics (European Environment Agency, 2018; Ormerod et al., 2010; Schinegger et al., 2016).

Mediterranean rivers are characterised by strong seasonality, with severe flood and drought periods, that increases their susceptibility to stressors (Colin et al., 2016; Gasith and Resh, 1999). Flow regime is the primary driving force of biological functions in Mediterranean rivers systems (Brown and Ford, 2002; Pool et al., 2010). Due to the scarcity of water resources, the European Mediterranean regions have a long history of flow regulation (Hooke, 2006). As a consequence, compared to other climatic regions, Mediterranean rivers suffer more severe human impacts (Clavero et al., 2010; Ilhéu et al., 2014; Maceda-Veiga, 2013; Radinger et al., 2018). For example, the southern Iberian Peninsula has a millennial history of human alterations that coexist with the Mediterranean character of its river systems (Bonada and Resh, 2013; Grindlay et al., 2011).

Due to their strong seasonality and harsh environmental conditions, fish assemblages of Mediterranean rivers are naturally characterised by low species richness with a high level of endemism (Poff and Allan, 1995; Tedesco et al., 2013). Furthermore, fish assemblages of river systems in the southern Iberian Peninsula have been severely altered by anthropogenic impacts that facilitated the establishment of multiple non-native species (Ilhéu et al., 2014; Rahel and Olden, 2008; Schinegger et al., 2016). As a consequence, fish assemblages in these rivers tend to be dominated by non-native species (Clavero et al., 2013).

During recent decade new approaches of functional diversity measures have been developed that more accurately reflect diverse facets about species roles inside ecosystems and hence, assemblage functional structure (Maire et al., 2015; Mouillot et al., 2013; Villéger et al., 2017). Therefore, the trait-based approach is recognised as a better approximation than classic diversity indices to assess dynamics of species assemblages (Petchey and Gaston, 2006; Villéger et al., 2010). Stress factors act as a filter of functional traits (Poff, 1997) and the environment–trait relationships suggest that each stress factor govern a particular set of traits (McGill et al., 2006). Previous studies have evaluated the effect of multiple stressors on functional traits of freshwater biota (Oliveira et al., 2012; Schinegger et al., 2016). Recent studies have documented significant relationship between flow alteration and functional diversity of macroinvertebrates in highly regulated Mediterranean rivers (Belmar et al., 2019; Laini et al., 2019; Suárez et al., 2016). However, functional diversity indices for fish assemblages in Mediterranean rivers are still poorly developed and have not been previously applied in highly regulated systems of this climatic zone (Colin et al., 2018; Pool et al., 2010).

The Segura River (main stem and its tributaries) is a Mediterranean river basin located in the southern Iberian Peninsula characterised by strong climatic and altitudinal gradient as well as significant flow regime heterogeneity (Belmar et al., 2011; Grindlay et al., 2011). These strong gradients and heterogeneity are expected to affect the structure and composition of its macroinvertebrate and fish assemblages (Bruno et al. 2014; Oliva-Paterna et al. 2014). In addition to natural gradients, the Segura River is also strongly affected by human activities such as withdrawal of water for human consumption, irrigation as well as hydropower generation (Belmar et al., 2013; Bruno et al., 2014). Indeed, the Segura River is one of the most severely regulated Mediterranean river systems (Grindlay et al., 2011). The present day fish assemblage of the Segura River is species poor (15 species) and dominated by cyprinids: two native and five non-natives species (Oliva-Paterna et al., 2019, 2014). Therefore, the Segura River offers an opportunity to evaluate the effects of multiple stressors on fish functional diversity in a

Mediterranean river basin. Functional indices such as specialisation, originality and entropy have been reported to be excellent indicators of ecological status of fish assemblages in species poor systems similar to the Segura River (Colin et al., 2018; Maire et al., 2015).

The objective of this study was to assess the response of three functional diversity indices (functional specialisation, originality and entropy) as well as classic taxonomic indices (species richness and abundance) of the Segura River fish assemblage to abiotic and biotic stressors. Furthermore, we identify specific species traits that are filtered by selected stressors. We hypothesised that the interplay of flow regime alteration and other stress factors result in functional homogenisation of fish assemblages within the system. Differential responses of functional diversity indices, species richness and abundances were expected because, despite the increase in species richness and abundance, fish assemblage could suffer a decrease in functional diversity due to the addition of equivalent species. Understanding of mechanisms in which stressors shape fish assemblages in Mediterranean rivers would inform management actions of these river systems to conserve and/or rehabilitate their ecological function in concordance with the EU's Water Framework Directive.

1.2 Materials and methods

1.2.1 Study area and sampling design

The study was conducted in the Segura River, placed in the south-east of the Iberian Peninsula. This river basin drains an area of 18,870 km^2 and is located in semi-arid climatic zone (Figure 1.1). A range of economic activities has been developed within the Segura River that is primarily related to agriculture. As a result, irrigation is responsible for 90% of water demands and constitutes the main anthropogenic pressure on the river. The regulation capacity by dams is approximately 1200 hm^3 (33 dams with storage capacity higher than 1 hm^3). Furthermore, since 1979 there is an inter-basin water transfer of approximately 350 hm^{3}year^{-1} from the Tajo River to the Mundo River, the main tributary of the Segura River (CHS, 2013; Grindlay et al., 2011). This situation caused significant modification of natural flow regime (Piqué et al., 2016) and severe degradation of riverine habitats in many reaches within the system (Belmar et al., 2013; Bruno et al., 2014). Furthermore, agricultural and urban activities are the primarily sources of pollutants discharged into the Segura River (CHS, 2015). These pollutants are dominated by nutrients (phosphates and nitrates) that enter into the river from artificially fertilised agricultural land (Pellicer-Martínez and Martínez-Paz, 2018). Consequently, the Segura River is considered as one of the most severely regulated Mediterranean river basins in Europe (Grindlay et al., 2011).

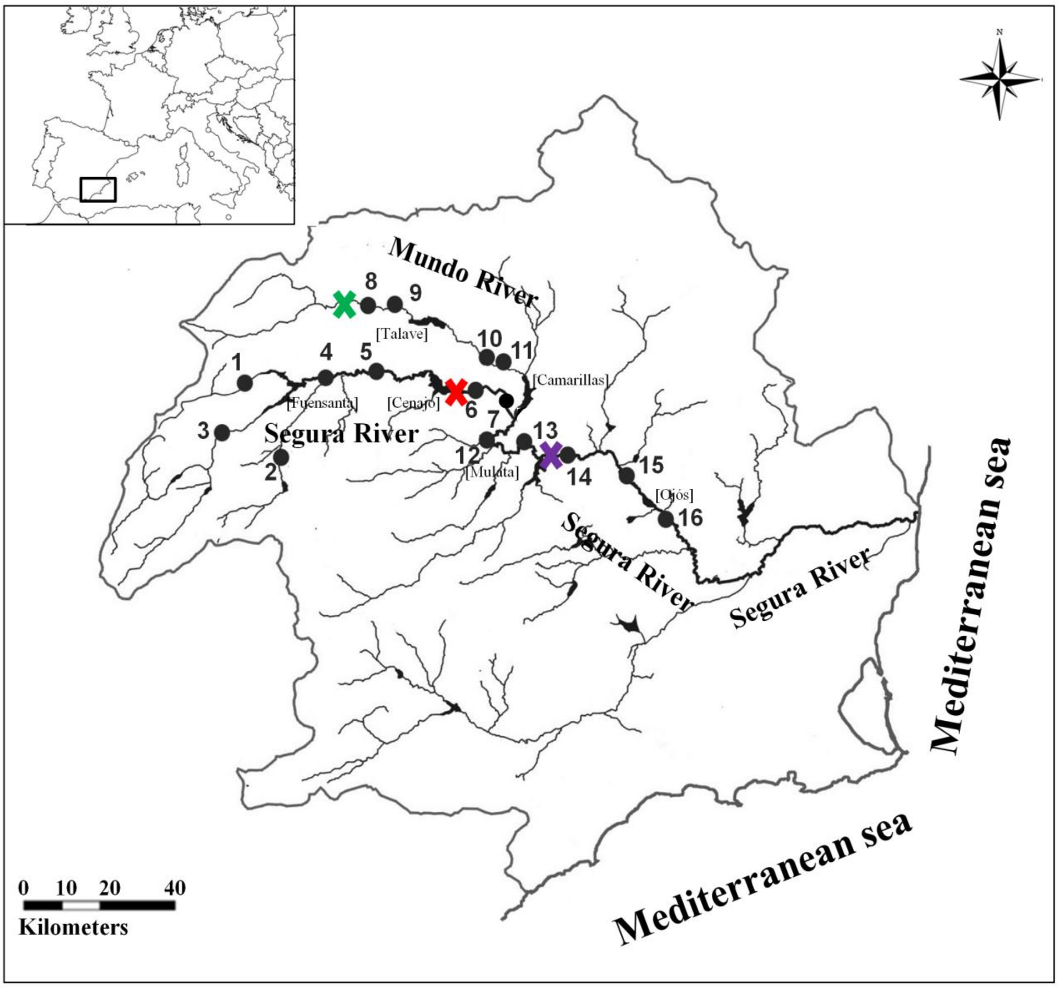

Figure 1.1 Study area. Sampling sites (●) (II) in the Segura River system and its location in the south-east of the Iberian Peninsula. Names of large reservoirs are indicated in parentheses []. The coloured **X** represents three gauging stations where the flow patterns are shown in figure 1.2.

We sampled fish assemblages in 16 sites across the Segura River (Figure 1.1). The altitude of the study area ranged between 112 and 809 m.a.s.l., water conductivity between 316 and 1157 µs cm^{-1}, and the mean annual water temperature between 12.9 and 18.4 ºC. Sampling sites were selected to reflect contrasting hydrological regimes. Areas representing the most natural flow regime (e.g. sampling sites 3, 8 and 9; Figure 1.1) were characterised by a strong seasonal variation, with droughts during summer and high flow picks in a short time period in spring and autumn (Figure 1.2a). A range of impacts was imposed on the natural flow regime in the study area (Amat-Trigo, 2018; Amat-Trigo et al., 2016). All impacted reaches are characterised by an inversion in the seasonality of the natural flow pattern (high flow levels in spring-summer and low levels in autumn-winter) as consequence of agricultural water demand. Furthermore, impacted reaches can be represented by two extremes: reaches placed just downstream of Cenajo reservoir (sampling sites 6 and 7; Figure 1.1) with high level of contingency (Impact 1, Figure 1.2b) and reaches characterised by stable flow regime and high level of base flow throughout the year (e.g. sampling sites 14 and 16, Figure 1.1; impact 2, Figure 1.2c).

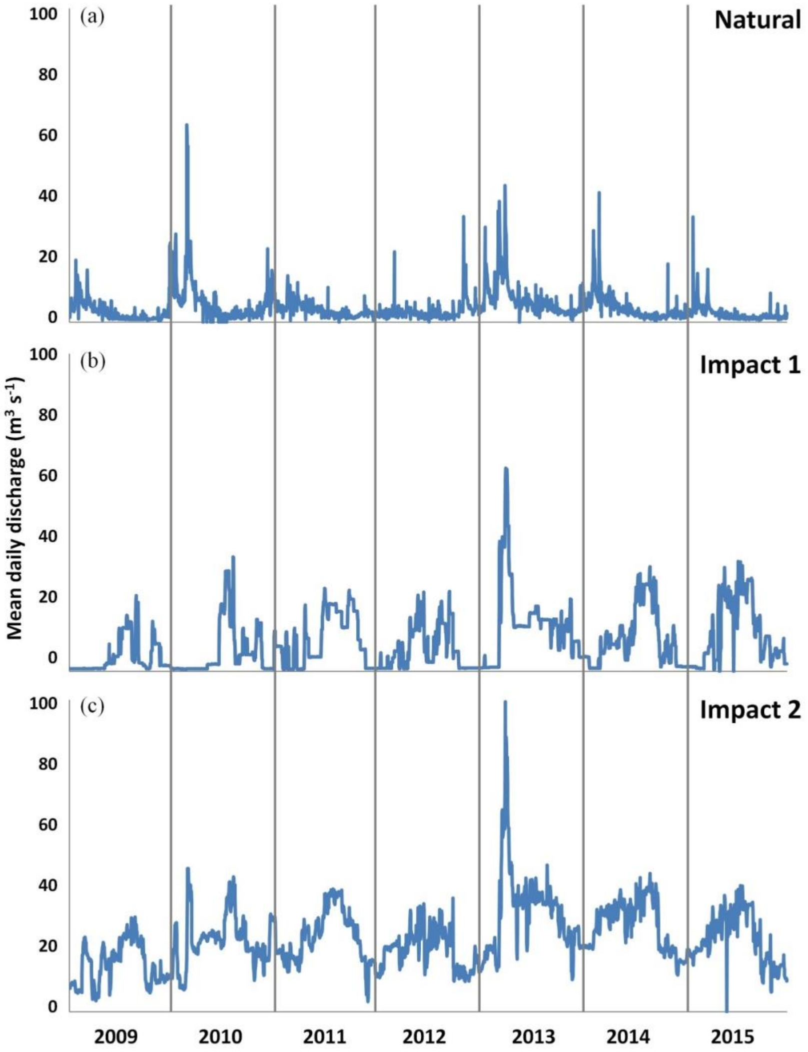

Figure 1.2 Inter-annual flow variations (mean daily discharge; m^{-3}s^{-1}) during the study period at three representative fluvial sectors in the study area. (a) represents the most natural flow regime at the upper part of the Mundo River (data obtained from gauging station marked as **X**; Figure 1.1), (b) impact 1 just downstream of the Cenajo reservoir (data obtained from **X**; Figure 1.1) and (c) impact 2 downstream of the La Mulata dam (data obtained from **X**; Figure 1.1). Flow discharge data were obtained from the Segura Hydrographic Confederation.

1.2.2 Environmental variables

Ten environmental variables and gradient descriptors were measured at each sampling site (Table 1.1): Ecological status according to categorisation of the EU Water Framework Directive, Fluvial Habitat Index (IHF) (Pardo et al., 2002), Riparian Quality index (RQI) (González del Tánago and García de Jalón, 2011) and altitude (CHS, 2007). Free reach (length of reach available for free movement) and Longitudinal Connectivity Index (ICL) (González Fernández et al., 2011) were calculated based on data obtained from on-line data bases from the Segura Hydrographic Confederation (CHS, https://www.chsegura.es/chs/cuenca/restauracionderios/obstaculos/visorjs.html; accessed September 2018). Daily river discharge, water conductivity and water temperature were also obtained from the Segura Hydrographic Confederation on-line data bases and were collected at gauging stations distributed throughout the study area: six stations located in the main stem of the Segura River and two in the Mundo River (CHS, https://www.chsegura.es/chs/cuenca/redesdecontrol/estadisticashidrologicas/; accessed September 2018). Mean monthly values of conductivity and water temperature were calculated for 2009, 2010, 2013, 2014 and 2015. Mean daily discharge data ($m^3 s^{-1}$) for a period between 1994 and 2015 were used to calculate Mean Daily Base Flow (MDBF, base flow component of the hydrograph divided by the number of days of the record) and flow variability (daily range between low Q_{10} and high Q_{90} discharge divided by median discharge). These two metrics were calculated using the Time Series Analysis TSA of the River Analysis Package (RAP version 3.0.7) (Marsh et al., 2003).

Table 1.1 Environmental variables measured to describe the study area.

Environmental variables	Description
Ecological status	Ecological status categorised as: 1 = high; 2 = good; 3 = moderate; 4 = poor.
IHF	Fluvial Habitat Index (%).
RQI	Riparian Quality Index (%).
Altitude	Meters above sea level (m).
Free reach	Length of the reach between non-passable barriers (km).
ICL	Longitudinal Connectivity Index. (Low values indicate higher connectivity).
Conductivity	Mean monthly water conductivity ($\mu s\ cm^{-1}$).
Water temperature	Mean monthly water temperature (ºC).
MDBF	Mean daily base flow (total base flow component of the hydrograph divided by the number of days of the record; $m^3 s^{-1}$).
Flow variability	Daily flow variability. Range between $Q_{10\%}$ and $Q_{90\%}$ divided by median value. (($Q_{10\%}$ - $Q_{90\%}$)/median).

1.2.3 Fish sampling

Fish were caught in each sampling site by electro-fishing (1800W generator, working voltage between 200 and 350V, 2–3 A), following the CEN standard protocol (CEN, 2003). Low population connectivity between fish captured in adjacent sampling sites was assumed (based on biology of each species, significant distance between sites and presence of dams and weirs), so each site was considered as an independent fish assemblage. Fish were sampled in wadable sections of 100 m blocked with nets that acted as barriers. Fishing at each occasion took 30-45 minutes. Sampling was performed during October-November in two periods of consecutive years (2009-2010 and 2013-2015). Collected fish were anaesthetised using clove oil and subsequently identified to species level and counted. Each species was classified as native or non-native (Table 1.2). After measurements, all native fish individuals were returned to their habitat in the river. Non-native individuals were sacrificed by overdose of anaesthetic and preserved in 10% buffer formaldehyde solution. Catch per Unit of Effort (CPUE) was calculated for each sampling site as number of fish caught per hour and 0.1 ha.

Table 1.2 Species captured in this study (N: native, E: exotic to the Segura River basin, T: translocated from Iberian basins). Common names for Iberian species according to Leunda et al. (2009).

Family	Specie	Code	Common name	Origin
Cyprinidae	*Alburnus alburnus* (Linnaeus, 1758)	Aa	Bleak	E
Cyprinidae	*Cyprinus carpio* Linnaeus, 1758	Cc	Common carp	E
Cobitidae	*Cobitis paludica* (de Buen, 1930)	Cp	Southern Iberian spined-loach	E (T)
Esocidae	*Esox lucius* Linnaeus, 1758	El	Northern pike	E
Cyprinidae	*Gobio lozanoi* Doadrio & Madeira, 2004	Gl	Pyrenean gudgeon	E (T)
Centrarchidae	*Lepomis gibbosus* (Linnaeus, 1758)	Lg	Pumpkinseed	E
Cyprinidae	*Luciusbarbus sclateri* Günther, 1868	Ls	Southern Iberian barbel	N
Centrarchidae	*Micropterus salmoides* (Lacépède, 1802)	Ms	Largemouth black bass	E
Salmonidae	*Oncorhynchus mykiss* (Walbaum, 1792)	Om	Rainbow trout	E
Cyprinidae	*Pseudochondrostoma polylepis* (Steindachner, 1864)	Pp	Iberian straight-mouth nase	E (T)
Percidae	*Sander luciusperca* (Linnaeus, 1758)	Sl	Pike perch	E
Cyprinidae	*Squalius pyrenaicus* (Günther, 1868)	Sp	Southern Iberian chub	N
Salmonidae	*Salmo trutta* Linnaeus, 1758	St	Brown trout	N

1.2.4 Functional diversity characterisation

Biological and ecological features of species were used to describe the functional structure of the fish assemblage (Villéger et al., 2010). We used nine functional traits related with eco-morphological and physiological features to assess the role of each species in the assemblage. We used available on-line data bases (Froese and Pauly, 2019; SIBIC 2021), and also literature (Doadrio, 2002; Fame Consortium, 2004; Kottelat and Freyhof, 2007) to define functional traits at the regional scale (Table 1.3). By combining relevant functional traits, it is possible to define a multidimensional functional space and to characterise the functional structure of fish assemblages. In order to evaluate the response of fish assemblages and their functional structure to environmental gradients, a set of complementary functional indices should be used (Mouillot et al., 2013). Therefore, based on previous studies in species poor systems (Colin et al., 2018), we used four functional diversity indices: functional specialisation (FSpe), originality (FOri), dispersion (FDis) and entropy (FEnt). In addition, we calculated species richness and total abundance to contrast functional diversity indices with traditional measures of the assemblage diversity.

Functional diversity indices (FSpe, FOri, FDis, and FEnt) were calculated based on forth-root transformed CPUE data for each site. These analyses were performed in R using the packages *ape, cluster, geometry* and *vegan.* Subsequently, Spearman correlations among diversity indices, species richness and total abundance were tested. FDis was highly correlated with FEnt (r = 0.94, p = <0.001) and, therefore, FDis was excluded from further analyses.

Table 1.3 Functional traits selected to describe the fish assemblage in the study area.

Trait	Data type	Categories	Categorical values
Maximum length	Continuous		
Maximum age	Continuous		
Fecundity (egg number)	Ordinal	< 2000	1
		2000 -10000	2
		>10000	3
Reproductive strategy	Ordinal	No spawning	1
		Unique event	2
		Fractional spawning	3
		Multiple spawning	4
Trophic guilds	Ordinal	Omnivore	1
		Invertivore	2
		Piscivore	3
Mesohabitat metric	Ordinal	Rheophilic	1
		Limnophilic	2
		Eurytopic	3
Vertical position	Ordinal	Benthic	1
		Benthopelagic	2
		Pelagic	3
Macrohabitat preferences	Ordinal	Up-stream	1
		Down-stream	2
		All range	3
Migratory behaviour	Ordinal	Non-migratory	1
		Potamodrome	2

1.2.5 Effects of stressors on functional diversity

We used general linear models (GLM) to test the effect of abiotic and biotic stress factors on functional diversity indices (FSpe, FOri and FEnt), species richness and abundance. These analyses were performed with *glm* function in R package *car*. Abiotic stress factors used in GLM models were obtained by reduction of environmental variables and gradient descriptors (ecological status, IHF, RQI, altitude, free reach, ICL, conductivity, water temperature, MDBF and flow variability (Table 1.1)) by Principal Component Analysis (PCA) method with Varimax rotation (Quinn and Keough, 2002). PCA was conducted using transformed values (log for numeric and arcsin square-root for percentage variables) in R using the package *psych*. Principal Component (PC) axes scores were used as different abiotic stress factors that could have effect on diversity metrics. Non-native species were considered as biotic stress factor and were expressed as the ratio of non-native species (non-native abundance/total abundance). Furthermore, sampling year was included as a factor to test the influence of different sampling periods on the results. To test relationships between the stressors and traits we carried out a RLQ approach to find the contribution of each functional trait in the fish assemblage. This method links information about species traits, environmental variables and species abundance by means of an ordination matrix (Dray et al., 2014). This analysis was computed in R using the package *ade4.*

1.3 Results

1.3.1 Stress factors

Three first PC axes from PCA analysis explained 80.2% of the total variation (Table 1.4) and were strongly associated with: habitat alteration and fragmentation (PC1), longitudinal gradient (PC2) and flow regime alteration (PC3). Habitat alteration and fragmentation (PC1) was associated with low ecological status, high values of conductivity and low RQI, as well as reduced connectivity (high values of ICL and short free reach). The longitudinal gradient (PC2) was associated with high altitude, low water temperature and high values of IHF (better fluvial habitat condition). Finally, flow regime alteration (PC3) was associated with flow variability and the magnitude of base flow (MDBF).

Table 1.4 Loadings and proportions of variance for each principal component axis (PC) extracted from environmental variables by PCA with Varimax rotation. Loadings > 0.60 are marked in bold.

Attribute	PC1	PC2	PC3
Ecological status	**0.820**	- 0.397	-
Fluvial Habitat Index (IHF)	0.141	**0.926**	0.127
Riparian quality index (RQI)	- 0.543	0.411	- 0.394
Altitude	- 0.416	**0.760**	-
Free reach	**- 0.913**	-	0.160
Longitudinal connectivity (ICL)	**0.867**	-	-
Conductivity	**0.740**	- 0.435	0.449
Water temperature	0.288	**- 0.677**	0.360
MDBF	-	- 0.588	**0.618**
Flow variability	- 0.103	-	**0.973**
Proportion of variance	0.339	0.276	0.187

1.3.2 Fish assemblage and functional diversity

A total of three native and ten non-native species belonging to six families was registered during the study (Table 1.2), with a range of species richness per site between two and nine. All sampling sites showed presence of non-native species and the ratio of non-native ranged between 0.21 and 1. *Luciobarbus sclateri* was the only native species present in the entire study area (16 sampling sites). Four species added ~95% of total CPUE. The most abundant species was *L. sclateri* (~36% of the total CPUE), followed by *Gobio lozanoi* (~30%), *Alburnus alburnus* (~19%) and *Pseudochondrostoma polylepis* (~10%). Functional diversity indices showed strong variation among sampling sites, FSpe ranged between 0.58 and 0.83, FOri between 0.48 and 0.92, whereas FEnt ranged between 1.24 and 2.17. Species richness and abundance were significantly correlated ($r = 0.82$, $p < 0.001$), and both were negatively correlated with FSpe: ($r = -0.84$, $p < 0.001$ for species richness and $r = -0.72$, $p < 0.001$ for abundance).

1.3.3 Effects of environmental stressors on species richness, abundance and functional diversity

Table 1.5 shows significant relationships among stress factors and each diversity indices, species richness and abundance obtained from the GLMs. Habitat alteration and fragmentation (PC1) were significantly linked to functional specialisation, species richness and abundance (Figure 1.3a, b, c). Specifically, habitat alteration and fragmentation were associated with functional specialisation decrease that was modulated by longitudinal gradient (PC1*PC2 interaction) (Figure 1.3a). In contrast, both species richness (Figure 1.3b) and abundance (Figure 1.3c) increased with the increment of habitat alteration and fragmentation. Longitudinal gradient by itself (PC2) had only marginally significant effects ($p < 0.1$) on functional entropy (Table 1.5) and was not included in Figure 1.3.

Table 1.5 Results of the general linear model analysis (GLM) for each diversity measure: functional diversity indices: specialisation (FSpe), originality (FOri) and entropy (FEnt), species richness and abundance, as a function of axes from environmental factors PCA: habitat alteration and fragmentation (PC1), longitudinal gradient (PC2) and flow regime alteration (PC3), non-native fish species ratio (Nn-ratio) and year. Significant probabilities ($p < 0.05$) are indicated in bold.

	χ^2	p
FSpe		
PC1	64.020	**<0.001**
PC2	22.255	**<0.001**
PC3	24.453	**<0.001**
Nn-ratio	21.355	**<0.001**
Year	22.376	**<0.001**
PC1*PC2	6.557	**0.010**
FOri		
PC3	4.971	**0.026**
Nn-ratio	40.368	**<0.001**
FEnt		
PC2	2.837	0.092
Species richness		
PC1	12.144	**<0.001**
PC3	4.454	**0.035**
Nn-ratio	4.204	**0.040**
Abundance		
PC1	38.019	**<0.001**
PC3	20.847	**<0.001**
Nn-ratio	8.989	**0.003**
Year	22.929	**<0.001**

Flow regime alteration (PC3) was significantly linked to functional specialisation, originality, species richness and abundance (Figure 1.3d, e, f, g). Sampling sites with more natural flow regime (Figure 1.2a) were located at intermediate values of the PC3 in the ordination space and correspond mainly to headwaters (Figure 1.1). The highest values of the PC3 reflect an increase in stability and base flow (impact 2; Figure 1.2c) and correspond to downstream sampling sites such as 14 and 16 (Figure 1.1). These high values of the PC3 were associated with decreased functional specialisation (Figure 1.3d) and originality (Figure 1.3e), but increased species richness (Figure 1.3f) and abundance (Figure 1.3g). The lowest values of the PC3 correspond to a decrease in flow stability and base flow (impact 1; Figure 1.2b), and correspond to sampling sites placed downstream Cenajo reservoir (Figure 1.1). These low values of the PC3 were associated with increased functional specialisation and originality but decreased species richness and abundance.

The ratio of non-native species was significantly linked to functional specialisation, originality, species richness and abundance (Figure 1.3h, i, j, k). Specifically, functional specialisation (Figure 1.3h) and originality (Figure 1.3i) significantly decreased with increase of the ratio of non-native species. Finally, sampling year was associated with functional specialisation and abundance (Figure 1.4). During the study period functional specialisation increased (Figure 1.4a) and abundance decreased (Figure 1.4b). Furthermore, we can observe greater differences between two sampling periods (2009-2010 and 2013-2014-2015) than between years within the same period; however these differences were not statistically significant.

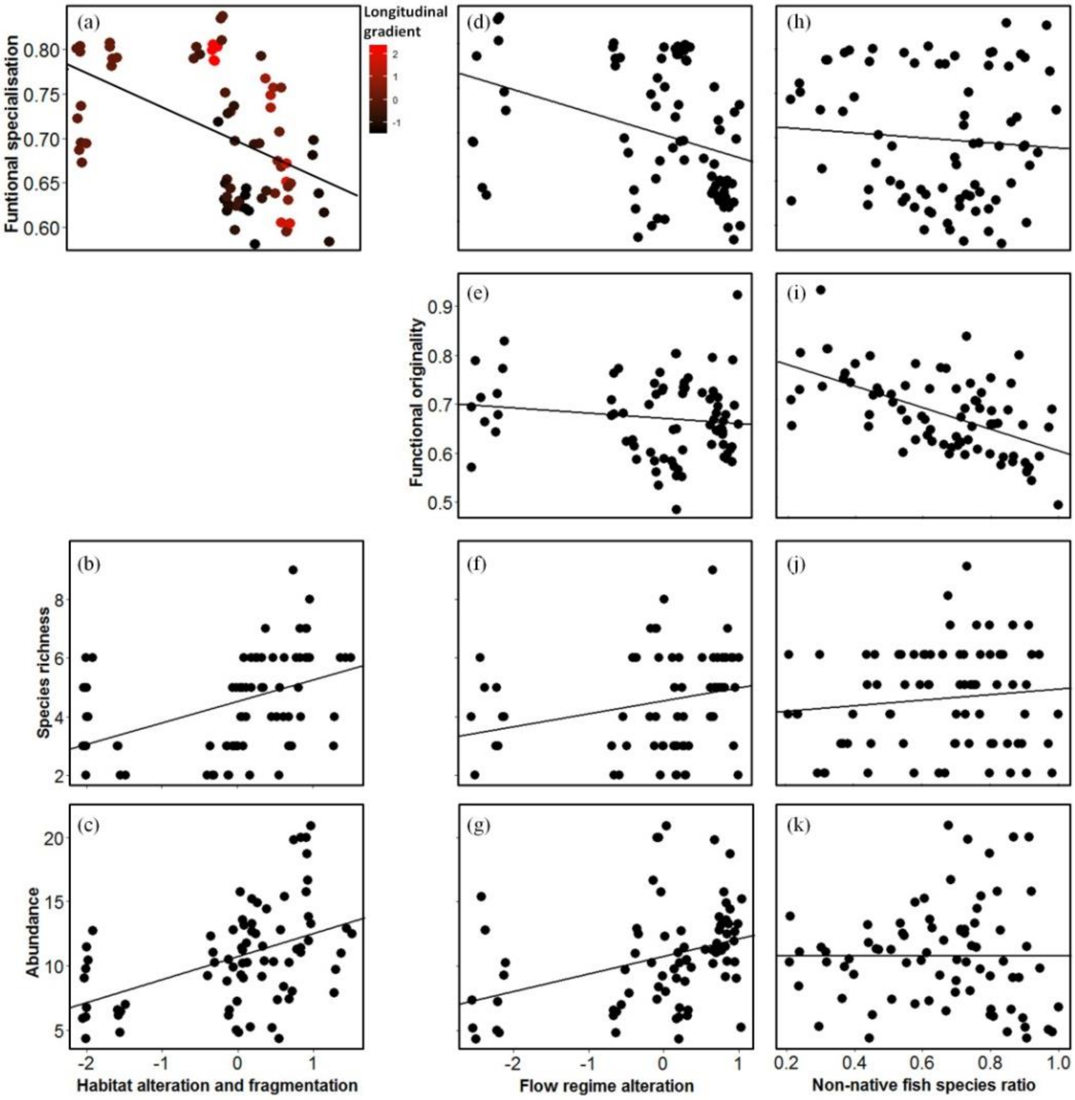

Figure 1.3 Significant relationships among stress factors and diversity measures (GLM $p < 0.05$). Effect of habitat alteration and fragmentation (PC1) on functional specialisation (a), species richness (b) and abundance (c); effect of flow regime alteration (PC3) on functional specialisation (d), functional originality (e), species richness (f) and abundance (g); effect of the presence of non-native fish species (non-native ratio) on functional specialisation (h), functional originality (i), species richness (j) and abundance (k).

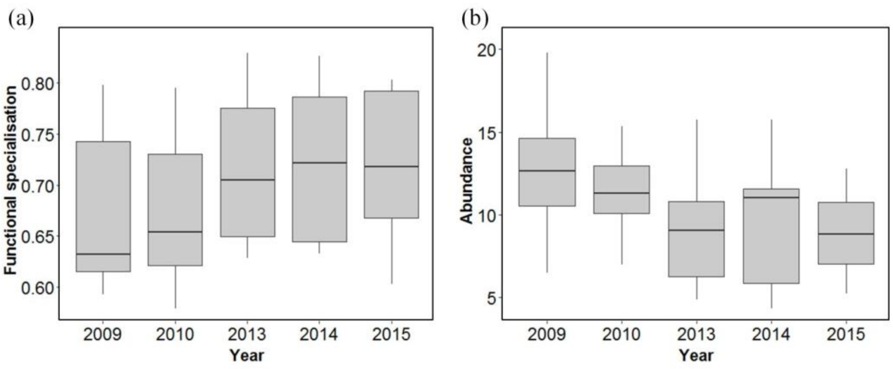

Figure 1.4 Temporal variations of functional specialisation (a) and abundance (b) during the study period.

1.3.4 Effects of environmental stressors on specific functional traits

We found marked associations of stressors and specific functional traits (Figure 1.5). Habitat alteration and loss of connectivity seemed to give advantage to fractional spawners and eurytopic tolerant species with a wide range of habitats. In contrast, reaches with better habitat conditions placed at higher altitudes, were mainly inhabited by headwater species with unique spawning event per year and low fecundity. Furthermore, fish assemblages with high number of non-native species were associated with high piscivory and high fecundity, whereas limnophilic species seemed to be favoured at downstream reaches with highly altered flow regime.

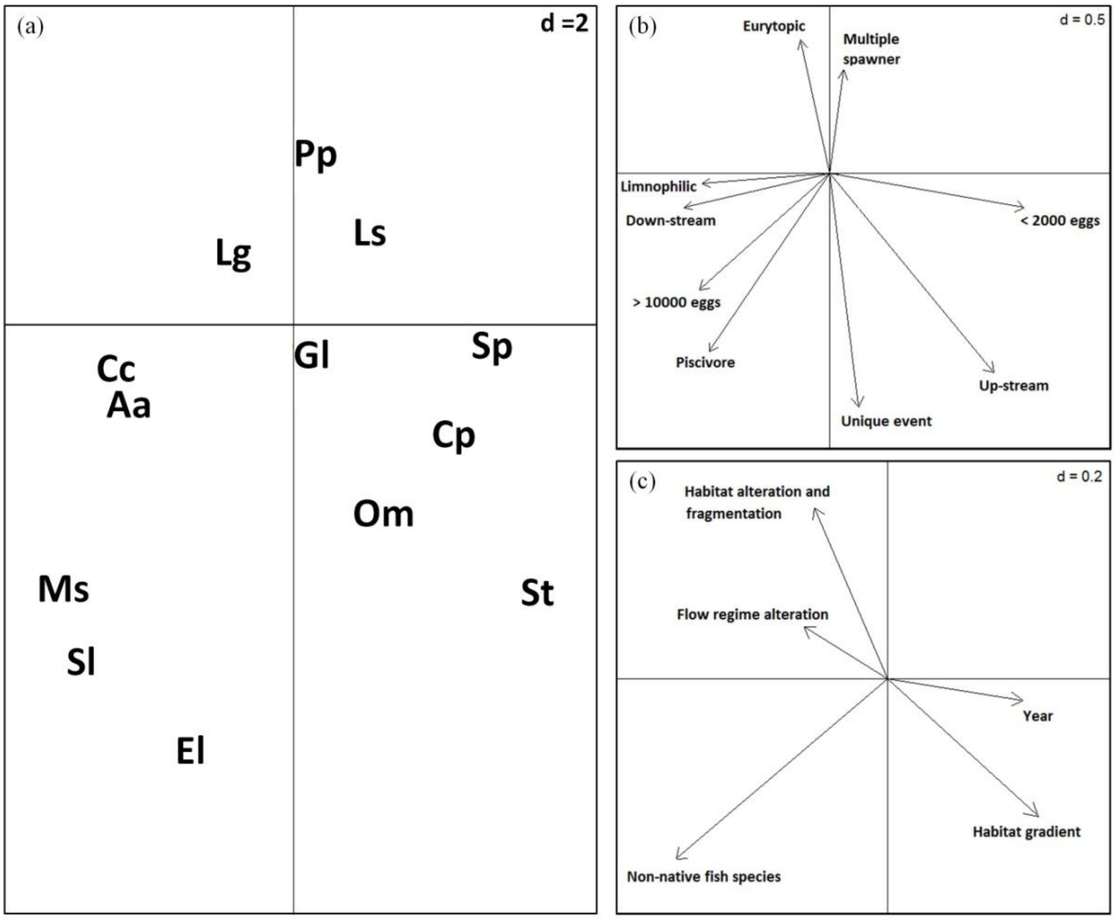

Figure 1.5 RLQ analysis results of the first two axes: (a) scores of the species (species codes in Table 1.1); (b) traits coefficients (description in Table 1.2) and (c) factors coefficients: habitat alteration and fragmentation (PC1), longitudinal gradient (PC2), flow regime alteration (PC3).

1.4 Discussion

This study assessed the effect of multiple stressors (abiotic and biotic) on fish assemblage functional diversity in a strongly regulated Mediterranean river basin, and found complex effects of these stressors. A combination of species richness and abundance with trait-based indices allowed a more detailed approximation of the fish assemblage response to stressors than measures based solely on traditional richness indices.

We found significant effects of habitat alteration and fragmentation, flow regime alteration and the presence of non-native fish species on the fish assemblage structure. These stressors caused homogenisation of the fish assemblage (reflected as a decrease in functional specialisation and originality) despite increase in species richness and abundance. It is essential to recognise the interplay among stressors tested in this study. Indicators such as ecological status, IHF, RQI, conductivity, temperature and the presence of non-native fish species are strongly modulated by flow regime alteration and fragmentation (Dudgeon et al., 2006; Jackson et al., 2016; Strayer and Dudgeon, 2010). This governing effect of flow regime alteration and fragmentation was also documented previously for other stress factors not considered in this study such as pollution or nutrient enrichment (Karaouzas et al., 2018; Martínez-Fernández, et al., 2019; Segurado et al., 2018). The interplay of these stressors drives environmental variables that act as a “filter” selecting species with optimal traits to inhabit areas with certain environmental characteristics (Poff, 1997). We found no correlation between the natural longitudinal gradient of the river and fish assemblages (only marginal effects on functional entropy). Headwaters are often characterised by more natural conditions compared to lower reaches within river systems because they are less accessible for human activities (Hughes et al., 2010). Easier access to water resources in lower reaches often implies upstream to downstream gradient of increasing stressors and degradation of river systems (Bruno et al., 2014; Fierro et al., 2019; Radinger et al., 2018; Tedesco et al., 2009). The lack of longitudinal gradient effect on the most of diversity measures is an effect of severe alterations of the Segura River along its entire gradient including headwaters (Belmar et al., 2013; Grindlay et al., 2011).

1.4.1 Effects of abiotic stressors

Our results showed that flow alteration with physical habitat, water quality and fragmentation govern fish assemblage structure in highly regulated Segura River. Flow regime alteration is the main stressor in river ecosystems (Bain et al., 1988; Poff and Zimmerman, 2010). In the Segura River, we observed an increase of species richness and abundance but the decrease of functional specialisation and originality in river reaches with high level of base flow and more stable hydrological conditions (Impact 2). Reduction of peaks of floods and droughts that are characteristic for Mediterranean river systems creates more lentic and homogeneous environments that favours non-native species (Alexandre et al., 2013a; Amat-Trigo, 2018; Brown and Bauer, 2010; Clavero et al., 2013). Abundant non-native species found in the Segura River, however, seem to be functionally similar hence lower functional specialisation and originality. The opposite pattern was observed in reaches placed downstream of a large reservoir (Cenajo Reservoir) used for water supply to agriculture characterised by severe reduction of base flow and marked inversion in the seasonal pattern of high and low flows (Impact 1) (Amat-Trigo, 2018; Amat-Trigo et al., 2016; Belmar et al., 2013; Piqué et al., 2016). Here species richness and abundance decreased, whereas functional specialisation and originality increased. High flow in spring and low in summer and autumn probably can only be tolerated by a small number of species (Alexandre et al., 2013a). Moreover, during summers these species are additionally affected by unnatural decrease in water temperature as a consequence of the operation of the dams with water discharges from the bottom of the reservoirs (Miranda et al., 2012), which could probably occur in the reach downstream Cenajo reservoir. Only widespread cyprinids (*L. sclateri* and *G. lozanoi*), *Lepomis gibossus* and *Esox lucius* were caught in these reaches. However, functional traits of these few species occupy extreme corners of functional space and drive high values of functional specialisation and originality in spite of low species richness and abundance (Mouillot et al., 2013).

These two contrasting outcomes of response in functional changes are an excellent example of how flow regime reinforces the structure of fish assemblages in river systems (Chaffin et al., 2016; Poff and Zimmerman, 2010). The governing role of flow regime in structuring fish assemblage is superimposed on physical habitat changes and water quality deterioration as well as fragmentation. Fragmentation and habitat loss cause local extinction of native species adapted to natural habitat conditions and connectivity (Benejam et al., 2015). These species, usually endemic in Mediterranean basins, support a specialist set of traits and their loss implies a decrease of functional specialisation (Brandl et al., 2016; Toussaint et al., 2018; Villéger et al., 2010). Conversely, non-native generalist species are often benefited by flow homogenisation and fragmentation in Mediterranean river systems (Clavero et al., 2013; Ribeiro et al., 2008).

1.4.2 Effects of non-native species

Fish assemblages in Mediterranean river systems have shown significant increase in species richness due to both historical and recent introductions of non-native fish (Koutsikos et al., 2019; Leunda, 2010; Martínez-Fernández, et al., 2019). Non-native fishes affect the ecological quality of rivers in multiple ways and may represent a serious threat to native species. Potential effects include spreading of pathogens and parasites, genetic alterations, competition and replacement of native fish, which usually have a more unique and extreme set of functional traits (Colin et al. 2018; Leunda 2010). The Segura River is characterised by an extremely low number of native species (a total of three, and only one widely distributed within the system). Similarly to other Mediterranean river basins, the Segura River has undergone significant fish richness changes in recent decades due to establishment of multiple non-native fish species throughout the entire basin (Oliva-Paterna et al., 2014). Addition of non-native species is expected to have severe effects in functional diversity of its assemblages in such species poor systems (Millardi et al., 2019; Toussaint et al., 2018). We reported significant decrease in functional specialisation and originality with increase of ratio of non-native species. Buisson et al. (2013) suggested that compared to other families of European fish, cyprinids are close to the average in the functional space and incorporate low originality to the fish assemblage. Indeed, fish assemblage of the Segura River was dominated by cyprinids, both native (i.e., *L. sclateri*) and non-native (i.e., *P. polypepis* and *G. lozani*) that share a similar set of functional traits and hence we observed inverse relationship of species richness and functional diversity indices.

1.5 Conclusions and management implications

Mediterranean fish species are adapted to strong seasonal variability and each species provides a different set of traits to form a fish assemblage with high level of specialisation and originality. Stressors caused alteration of fish assemblages by loss of native species and the establishment of non-native species in Mediterranean river basins (Ilhéu et al., 2014). The wide distribution of non-native species in the Segura River and adjacent Mediterranean rivers in the southern Iberian Peninsula makes actions aimed at their direct control or eradication very challenging. Knowledge on response of fish assemblage to different stressors may allow management of some these stressors to favour native fish. Removal of barriers to restore the natural flow regime is complicated when they are operational. Nevertheless, design flows to mimic natural flow regime patterns with Mediterranean rivers characteristics could provide environmental conditions that would favour native fish within the assemblage (Chen and Olden, 2017; Marchetti and Moyle, 2001; Poff, 1997). Mediterranean fish species are adapted to high flow variability and climate change is expected to increase frequency and magnitude of flood and droughts in Mediterranean climatic areas (IPCC, 2019). Therefore, flow management that allows recovery of native fish assemblages in Mediterranean river basins may increase climatic resilience of these ecosystems. The use of tools that consider functional traits of species as method to assess the effects of environmental alterations on fish biodiversity could improve and refine conservation measures to protect the Mediterranean fish fauna facing climate change.

Chapter 2

Population traits variability of a sentinel Iberian fish, *Luciobarbus sclateri* (Günther, 1868), in a highly regulated Mediterranean river

Abstract

Human pressures on water resources have been suggested as a driver of biological traits that induce changes in native fish populations. This study highlighted the interplay between environmental stress factors, mostly related to flow regulation, and the longitudinal river gradient in biological traits such as the growth, size structure and somatic condition of a sentinel fish, *Luciobarbus sclateri*. We found an increase in size-related metrics and somatic condition at population levels associated with downstream reaches, although fragmentation and habitat alteration, flow regime alteration and the abundance of non-native fish were also significantly involved in their variability. Age-related parameters and growth were only explained by flow regime alterations and the abundance of non-native fish species. The high plasticity observed in *L. sclateri* population traits suggests that this is a key factor in the species adaptability to resist in a strongly altered Mediterranean river basin. However, the interplay of multiple stressors plays an important role in fish population dynamics and could induce complex responses that may be essential for long-term monitoring in sentinel species.

2.1 Introduction

Freshwater ecosystems are considered among the most altered as a consequence of the historical pressure of human activities (Sabater et al., 2018; Srinivasan et al., 2012). Hydraulic management to take advantage of water resources and the effects of climate change are inducing quantitative and qualitative changes in river systems (Veldkamp et al., 2017; Vörösmarty et al., 2000). These changes imply hydro-morphological, chemical and biological alterations which affect the freshwater fauna (Dudgeon et al., 2006; Sabater et al., 2018). Hydraulic management is especially intense in Mediterranean regions where water resources are scarce (Hooke, 2006). Rivers in semi-arid regions, such as the Iberian Peninsula, are heavily impacted by the construction a large number of dams and weirs (Brink et al., 2018; Maceda-Veiga, 2013).

Mediterranean regions are characterized by marked seasonality and inter-annual variability with severe periods of floods and droughts (Gasith and Resh, 1999; Vidal-Abarca et al., 1992). The native freshwater fauna is adapted to such natural variability and displays great resistance and resilience (Alexandre et al., 2013a; Encina et al., 2006; Hershkovitz and Gasith, 2013); however, it is considered especially sensitive to human impacts (Kottelat and Freyhof, 2007; Ormerod, 2003). Human pressures are particularly severe in Mediterranean regions and they coincide with high natural variability, causing severe alterations to fluvial ecosystems (Bonada and Resh, 2013; Clavero et al., 2010; Maceda-Veiga, 2013). Despite the high adaptability of freshwater fauna, the expected increase in human impacts under future scenarios of global climate change could increase its vulnerability to such pressure, especially in Mediterranean regions (Bunn and Arthington, 2002; Hershkovitz and Gasith, 2013; Poff and Zimmerman, 2010).

The flow regime is considered one of the main driving forces of freshwater ecosystems, determining the structure and ecological dynamic of rivers (Lytle and Poff, 2004; Poff and Zimmerman, 2010). In the Iberian Peninsula, the alteration of the natural flow regime by dam regulation is one of the most important stress factors negatively affecting the native fish fauna (Belletti et al., 2020; Fornaroli et al., 2020; Hermoso and Clavero, 2011). The effects of flow regime alteration have been widely documented in Mediterranean fish populations (Alexandre et al., 2014a; Bernardo et al., 2003; Branco et al., 2017; Merciai et al., 2017a). In addition, flow management infrastructures cause loss of connectivity and habitat alterations related to the fragmentation process (e.g., increase in lentic habitats, changes in water quality), which could facilitate the establishment and spread of non-native fish species, altering the composition of the community (Ilhéu et al., 2014; Schinegger et al., 2016). However, the effects of these stress factors have been poorly studied in Mediterranean rivers (Colin et al., 2018; Sánchez-Pérez et al., 2020; Segurado et al., 2018).

The Segura River basin is located in the south-east of the Iberian Peninsula and is characterized by a marked environmental variability and displays a wide variety of human impacts along its longitudinal gradient (Belmar et al., 2013; Bruno et al., 2014). Furthermore, this basin shows a well-documented range of impacts on the natural flow regime (Amat-Trigo, 2018; Amat-Trigo et al., 2016) and is considered one of the most regulated Mediterranean river systems (Grindlay et al., 2011). Therefore, the Segura River offers an opportunity to study the effect of multiple human-induced stressors along an intensively altered basin. The fish assemblage in the Segura River basin is characterized by a low number of species where *Luciobarbus sclateri* (Günther, 1868) appears to be dominant (Oliva-Paterna et al., 2014). This native species is an endemic potamodromous fish considered a sentinel species in the southern Iberian Peninsula (Encina et al., 2006), and its biology and ecology have been well documented (Encina and Granado-Lorencio, 1997; Herrera et al., 1988; Torralva, 1996), being the unique native fish widely distributed in the Segura River basin (Martínez-Morales et al., 2010). Therefore, *L. sclateri* populations in the Segura River could be considered a useful tool through which to assess the intra-specific variability along a wide longitudinal gradient strongly affected by environmental alterations mainly related to flow regulation. However, few authors have described the effects of multiple stressors on its populations, and nothing is known about its intra-specific variability along longitudinal gradients (De Miguel et al., 2016; Oliva-Paterna et al., 2003a; Torralva et al., 1997).

The main objective of the present study was to assess the variability of *L. sclateri* population traits in relation to different environmental stressors along the longitudinal gradient of a highly regulated river system. Our hypothesis was that *L. sclateri* would exhibit high phenotypic plasticity that enables populations to survive along the longitudinal gradient of a highly impacted river basin. *L. sclateri* has been evaluated as near threatened (NT) on a regional scale due to the intensification of human pressures which caused a decrease in habitat quality (mainly pollution and flow regime alteration) and an increase in the establishment and spread of several non-native fish species (Torralva et al., 2005). Furthermore, in recent years, a severe decline in *L. sclateri* populations has been documented in the Segura River basin (Oliva-Paterna et al., 2019, 2014). The use of different biological traits provides ecological insights into how populations respond to multiple stress factors, allowing us to understand how species' traits could predispose species to local extinctions (Bergerot et al., 2015) and to establish more successful management and recovery programs (Cooke et al., 2012; Maceda-Veiga, 2013).

2.2 Materials and methods

2.2.1 Study area and sampling design

This study was conducted in the Segura River basin, situated in the semi-arid Mediterranean climatic zone in the south-east of the Iberian Peninsula (Figure 2.1). This river basin drains an area of 18,870 km^2 and is characterized by a strong climatic and altitudinal gradient; and significant annual and inter-annual natural variation in the flow regime (Hooke, 2006). Multiple human activities —primarily related to agriculture, but also to electricity generation and human supply— have developed in the study area (Belmar et al., 2013; Bruno et al., 2014; Grindlay et al., 2011). Irrigation accounts for 90% of the water demand and is considered the main pressure on the water resources. A total of 33 dams (>10 m height and >1 hm^3 of reservoir) and 170 smaller obstacles exist along the longitudinal gradient of the river with a capacity of regulation of approximately 1200 hm^3. Since 1979, this basin has received an external water transfer from the Tajo River with an average of 350 hm^{3}year^{-1}, so the storage capacity increased by around 140% of the natural input (871 hm^{3}year^{-1}) (CHS, 2013; Grindlay et al., 2011). Furthermore, local agricultural practices add an artificial source of pollutant discharge (mainly phosphates and nitrates) into the river (CHS, 2015; Pellicer-Martínez and Martínez-Paz, 2018). As a result, the fluvial and riparian habitats of the Segura River basin have been severely altered (Belmar et al., 2011; Bruno et al., 2014), in addition to strong modifications in the natural flow regime (Piqué et al., 2016).

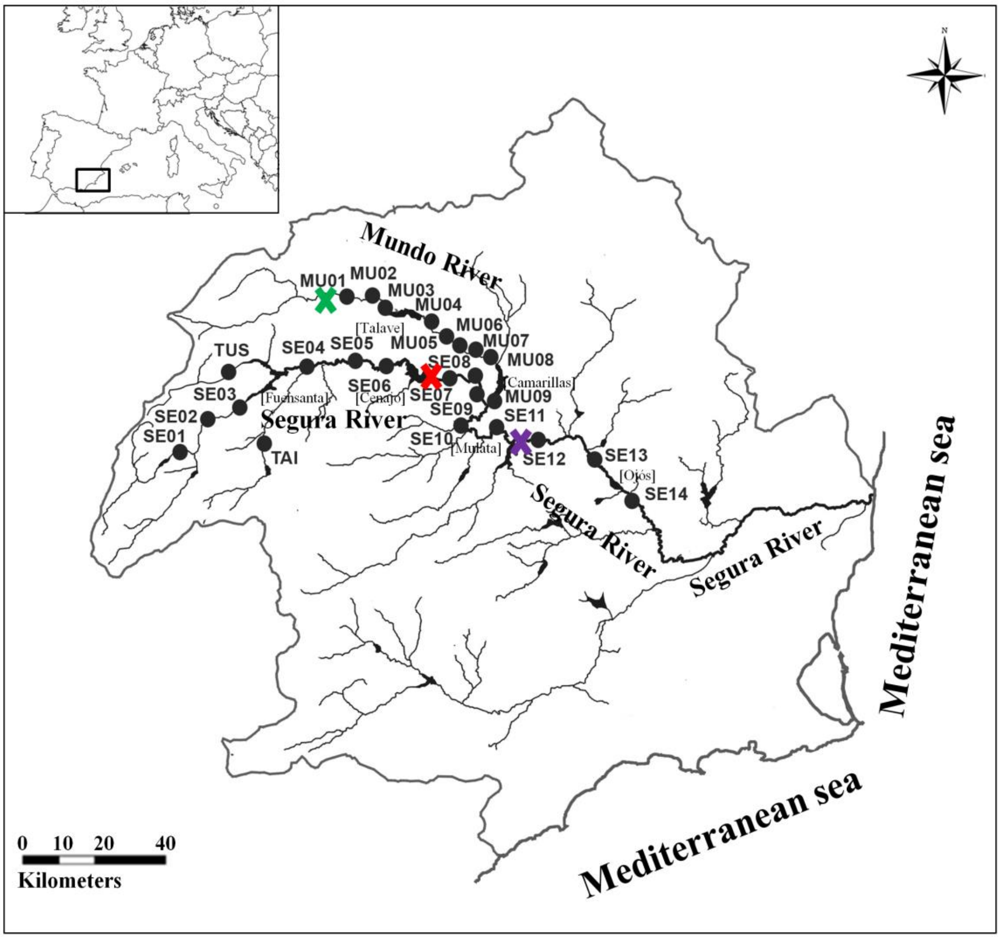

Figure 2.1 Study area. Location of the Segura River basin in the south-east of the Iberian Peninsula. Sampling sites are marked (●): TAI (Taibilla River), TUS (Tus River), MU (Mundo River) and SE (Segura River). Names of large reservoirs are between parentheses [].The coloured **X** represents three gauging stations where the flow patterns are shown in figure 2.2.

We sampled a total of 25 sites in fluvial reaches distributed along upstream–downstream gradients from the upper Segura main stem (SE) to the middle Segura (195 km), from the upper Mundo River (MU) to the Mundo–Segura confluence (51 km) and in two upper tributaries (Tus and Taibilla) (Figure 2.1). The range in altitude is 112–809 m.a.s.l. in the study area; the water conductivity ranged from 316 to 1303 µs cm^{-1} and the range of the mean annual water temperature was 13.9–16.9 °C during the study period. The distribution of sampling sites reflects the different hydrological flow regimes present in the study area. Sampling sites placed in areas with the most natural flow regime (e.g., MU01 and SE01; Figure 2.1) were characterized by a strong seasonal variation, alternating summer droughts and spring/autumn short-time flow peaks (Figure 2.2a). An impact gradient on the natural flow regime in the study area was described by Amat-Trigo (2018) and Amat-Trigo et al. (2016). Impacted areas exhibit a seasonal inversion of the natural flow pattern (high flow levels in spring and summer, low flow levels in autumn and winter) due to the water demand for agricultural practices. Furthermore, impacted areas can be characterized by two extreme flow impacts: reaches downstream of the Cenajo reservoir (sampling sites SE06, SE07 and SE09; Figure 2.1) showed a high level of contingency and low variability, but also low predictability (Impact 1, Figure 2.2b), while other reaches (e.g., sampling sites MU09, SE12 and SE13; Figure 2.1) were characterized by more stable and high levels of base flow throughout the year, high values of temperature and spell peaks, in addition to the inversion in flow seasonality (Impact 2, Figure 2.2c).

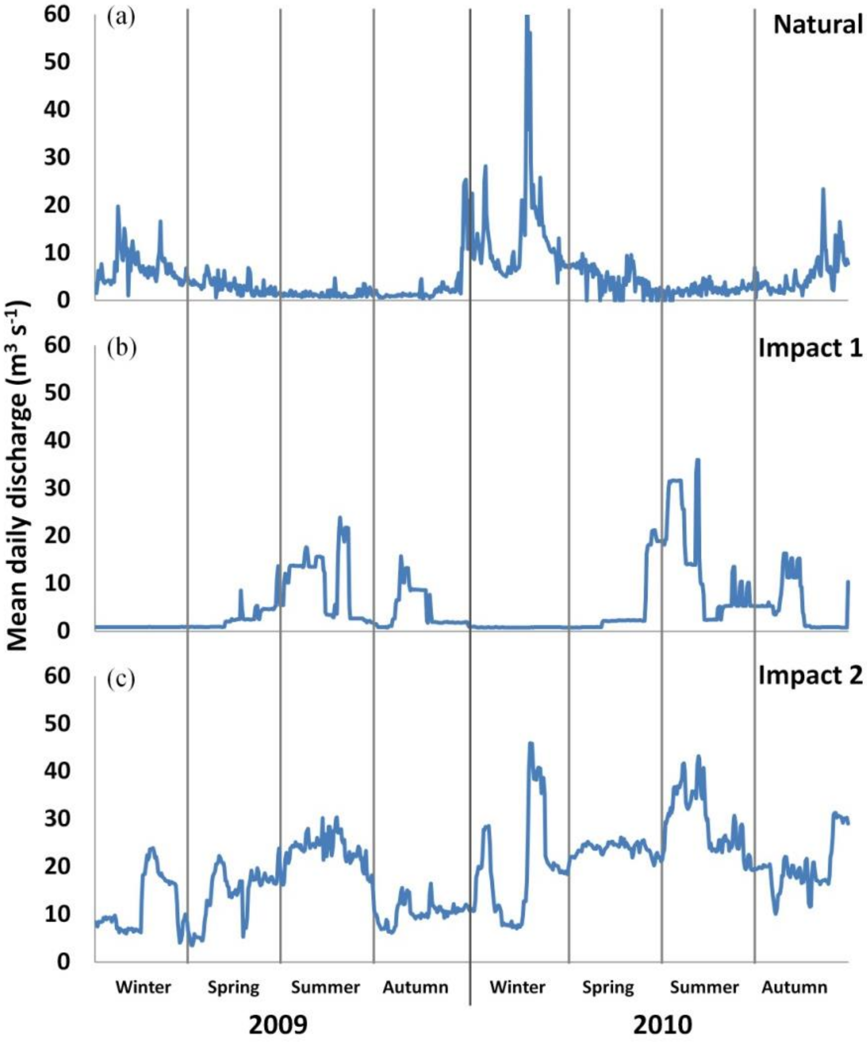

Figure 2.2 Flow variation (mean daily discharge; m^3 s^{-1}) during the study period at three representative fluvial sectors in the study area. (a) represents the most natural flow regime at the upper part of the Mundo River (data obtained from gauging station marked as X; Figure 2.1), (b) impact 1 just downstream of the Cenajo reservoir (data obtained from X; Figure 2.1) and (c) impact 2 downstream of the La Mulata dam (data obtained from X; Figure 2.1). Flow discharge data were obtained from the Segura Hydrographic Confederation.

The fish assemblage in the study area is composed of both native and non-native species (Oliva-Paterna et al., 2014). Non-native species are dominant (90% of total species richness) and *L. sclateri* is the only widely distributed native species in the study area. The most abundant species are cyprinids: the native *L. sclateri* and the non-native species *Pseudochondrostoma polylepis* (Steindachner, 1864), *Gobio lozanoi* (Doadrio and Madeira, 2004) and *Alburnus alburnus* (Linnaeus, 1758). The non-natives *Cyprinus carpio* (Linnaeus, 1758) and *Lepomis gibbosus* (Linnaeus, 1758) are locally abundant. Furthermore, the natives *Squalius pyrenaicus* (Günther, 1868) and *Salmo trutta* (Linnaeus, 1758), as well as the non-natives *Gambusia holbrooki* Girard 1859, *Oncorhynchus mykiss* (Walbaum, 1792), *Micropterus salmoides* (Lacépède, 1802), *Sander lucioperca* (Linnaeus, 1758) and *Esox lucius* (Linnaeus, 1758), are present in the study area.

2.2.2 Environmental variables

We described a total of ten environmental variables and gradient descriptors in the study area (Table 2.1). Water conductivity was measured in situ using a multi-parameter (340i WTW), and ecological status was assessed according to the EU Water Framework Directive, Fluvial Habitat Index (IHF) (Pardo et al., 2002), Riparian Quality Index (RQI) (González del Tánago and García de Jalón, 2011) and altitude (CHS, 2007). Longitudinal Connectivity Index (ICL) (González Fernández et al., 2011) and free reach (length of reach available for free movement) were calculated using databases from the official monitoring service of the Segura Hydrographic Confederation (CHS) (https://www.chsegura.es/chs/cuenca/restauracionderios/obstaculos/visorjs.html; accessed September 2018). Water temperature and daily river discharge data were also obtained from CHS databases with data from gauging stations distributed in the study area (https://www.chsegura.es/chs/cuenca/redesdecontrol/estadisticashidrologicas/; accessed September 2018). Mean monthly values of water temperature for 2009 and 2010 were calculated. Mean daily discharge ($m^3 s^{-1}$) over a 16-year period (1994–2010) was used to calculate the mean daily base flow (MDBF) as the total base flow component of the hydrograph divided by the number of recording days, and flow variability as the daily range between $Q_{10\%}$ and $Q_{90\%}$ discharge divided by the median value. These two flow metrics (MDBF and flow variability) were calculated using time series analysis (TSA) of the River Analysis Package (RAP version 3.0.7) (Marsh et al., 2003).

Table 2.1 Environmental variables and gradient descriptors measured or calculated at sampling sites. Altitude (meters above sea level), ecological status (categorized as: 1 = high; 2 = good; 3 = moderate; 4 = poor), Fluvial Habitat Index (IHF) and Riparian Quality Index (RQI) (%), conductivity (µS cm^{-1}), free reach (km), Longitudinal Connectivity Index (ICL), water temperature (°C), mean daily base flow (MDBF) (m^{3} s^{-1}) and flow variability ((Q$_{10\%}$ − Q$_{90\%}$)/median).

Sampling site	Altitude	Ecological status	IHF	RQI	Conductivity	Water temperature	Free reach	ICL	MDBF	Flow variability
MU01	560	2	72	55	602	14.7	4.13	255	1.59	−2.44
MU02	540	2	81	85	619	14.7	2.43	255	1.59	−2.44
MU03	520	2	81	85	619	14.7	5.39	255	1.59	−2.44
MU04	480	3	69	43	639	14.7	3.25	205	8.58	−1.71
MU05	460	3	75	53	674	14.7	2.02	205	8.58	−1.71
MU06	430	3	70	64	688	14.7	3.67	205	8.58	−1.71
MU07	396	3	72	46	724	14.7	3.79	205	8.58	−1.71
MU08	354	3	70	34	1303	14.7	2.29	205	8.58	−1.71
MU09	350	4	67	39	1109	15.4	3.54	138	14.04	−1.62
SE01	860	3	78	94	364	14. 3	25.88	84	2.91	−3.14
SE02	685	1	61	98	387	14.3	32.33	84	2.91	−3.14
SE03	491	1	63	87	390	14.3	12.58	84	2.91	−3.14
SE04	470	1	67	78	340	13.8	30.02	0	4.14	−2.58
SE05	452	1	68	80	401	13.8	30.02	0	4.14	−2.58
SE06	432	1	78	98	401	13.8	30.02	0	4.14	−2.58
SE07	363	2	67	77	447	14.1	7.73	126	1.77	−13.10
SE08	325	2	73	66	492	14.1	5.96	126	1.77	−13.10
SE09	306	2	58	69	579	14.1	1.7	126	1.77	−13.10
SE10	290	2	62	32	756	15.9	7.18	126	14.04	−1.62
SE11	260	3	57	44	803	15.9	10.64	90	14.04	−1.62
SE12	200	2	66	70	861	16.4	4.98	106	14.22	−1.55
SE13	148	2	58	45	1139	16.4	6.88	365	15.14	−1.47
SE14	112	4	64	36	1157	16.8	4.36	365	2.46	−3.31
TAI	640	2	61	43	566	14.7	15.67	58	-	-
TUS	809	1	84	65	432	14.5	12.81	76	1.59	−2.44

2.2.3 Fish sampling and population traits

Sites were sampled using electro-fishing (1800W generator, working voltage between 200 and 350 V, 2–3 A), following the CEN standard protocol (CEN, 2003). Each sampling site was considered an independent population, taking into account the distance between sampling sites, the presence of non-passable barriers (dams and weirs) and the biological characteristics of *L. sclateri*. Fish were collected in 100 m long wadable sections blocked by nets that acted as barriers. Fish sampling sessions were carried out in 30–45 min. Sampling was conducted during October–November 2009 to prevent the capture of spawning fish (Torralva et al., 1997) and to avoid variation in body condition due to gonad development (Encina and Granado-Lorencio, 1997; Oliva-Paterna et al., 2003b; Torralva et al., 1997). Fish manipulation was carried out following the European Union Directive 2010/63/UE on the protection of animals used for scientific purposes and it was not necessary to obtain authorization from the research ethics commission. In concordance with administrative permits, a total of 1529 specimens of *L. sclateri* were caught, processed in the field and returned to their habitat. Fork length (FL ± 0.1 cm) and weight (TW ± 0.1 g) were measured. A subsample of scales (611 specimens) was taken and cleaned later in a laboratory to determine the age according to Herrera et al. (1988) and Torralva et al. (1997).

L. sclateri populations were evaluated at sampling sites to assess intra-specific variability. The fish population traits we studied were relative abundance, size-related parameters, age-related parameters, relative growth rates and somatic condition. Relative abundance was measured as the number of *L. sclateri* individuals caught per hour (catch per unit effort, CPUE) in a standardized sample area without significant differences in habitat complexity and, thus, assuming that catch efficiency remains constant. Size-related parameters included mean, maximum and range of fork length (FL), and a size diversity index calculated as a Shannon-Wiener modification using the number of size classes grouped in 2 cm length ranges. Age-related parameters included mean, maximum and range of age, determined for a subsample of *L. sclateri* scales. Back-calculated lengths were estimated by the Fraser-Lee equation following the methodology used by Torralva et al. (1997) and Miñano et al. (2000) based on the counting and measuring of scales' annuli, and checked according to Musk et al. (2006). Proportions (%) of back-calculated lengths for each age class were obtained from the Walford method (Walford, 1946) and used to calculate the mean individual growth index (GI). Relative growth rates at the site level were estimated from the mean individual growth index (GI). The methodology used to calculate the growth index (GI) is detailed in Masó et al. (2016) and Amat-Trigo et al. (2019), which followed the Hickley and Dexter procedure (Hickley and Dexter, 1979). We used the mean values of GI at age 1 year, age 2 years and maturity (individuals older than 2 years) according to the age of maturity previously established for the species in the Segura River basin (Oliva-Paterna et al., 2003a, 2003b; Torralva et al., 1997). Somatic condition was expressed as predicted values of log-transformed weight (mean value at sampling site) obtained from the application of univariate analysis of covariance (ANCOVA) using total weight (WT) as the dependent variable and fork length (FL) as the covariate; differences in variation were tested by ANOVA and Tukey's HSD post hoc tests (Garcia-Berthou and Moreno-Amich, 1993). Individuals with an FL less than 75 mm were considered juveniles (Oliva-Paterna et al., 2003a). Bivariate relationships between population traits were analyzed using Spearman's rank correlations. Statistical analysis was performed with the SPSS software package v. 24.

2.2.4 Effect of environmental stress factors on population traits

We conducted a model selection analysis based on the Akaike Information Criterion with a correction for small samples sizes (AICc) (Burnham and Anderson, 2002) to determine the stressors associated with the variability in *L. sclateri* population traits. To establish model ranking, the MuMIn (Multi-Model Inference) R Package was used (Barton, 2018). Stress factors were obtained by the reduction in transformed environmental variables and gradient descriptors (log for numeric variables, arcsine square root for proportions) using principal component analysis (PCA) with Varimax rotation (Quinn and Keough, 2002) using R package *psych*. Spearman's rank correlation analysis was used to test the redundancy between the variables. Principal component axes scores were used as stressors. The ratio of the abundance of non-native fish species (abundance of non-native species/total abundance) was also considered a stressor. General linear model results (GLMs) for the best models (ΔAICc < 2.0) were used to describe the response of *L. sclateri* biological traits to the stress factors. These analyses were performed in the R statistical environment (Version 1.40.4).

2.3 Results

2.3.1 Relative abundance and population traits

The abundance of *L. sclateri* showed high spatial variation, ranging from 13.33 to 150.00 catches per unit of effort (CPUE, Table 2.2). The highest value was found in the Taibilla tributary (sampling site TAI) and the lowest was found at SE04, downstream of the Fuensanta reservoir (Figure 2.1).

The mean size fork length in the whole study area was 18.3 ± 6.55 cm FL, with the maximum value detected at MU03 (58.2 cm). The maximum validated age was 15+ years, with individuals this old detected at sampling sites SE04, SE07 and SE09; however, the mean age at the population level was 4.9 ± 1.43 years (Table 2.2). The population size/structure differed among sampling sites, although a polymodal distribution pattern was evident in most sampling sites along the longitudinal gradient (Figure 2.3). Both age and size parameters displayed lower values in headwaters (e.g. SE01 in Figure 2.3b and TUS in Figure 2.3c), with maximum fish sizes below 30 cm (FL), lower size ranges and lower size diversity index values (Table 2.2). The other sampling sites had specimens longer than 40 cm (FL), with higher values at sampling sites just downstream of non-passable obstacles (e.g., SE07, Figure 2.3b) and upstream of large reservoirs (e.g., MU03, Figure 2.3a).

The growth index (GI) displayed high variability for individuals of each age class between sampling sites (Table 2.2). The mean GI value at age 1 year across the study area was 66.74 ± 1.28, with a maximum value of 82.47 detected at sampling site SE01, while the mean value of GI at age 2 years was 39.34 ± 1.13, with a maximum of 53.52 at sampling site MU09. For mature fish, the mean GI value in the study area was 17.69 ± 1.57, with a maximum of 32.53 detected at site MU05. The lowest GI values were detected in the TUS tributary (age 1 year = 45.72; age 2 years = 19.26; mature = 0.03).

Somatic condition was higher in mature individuals and also showed greater variability among sampling sites (Table 2.2). The mean of predicted values of log-transformed weight for mature individuals was 1.87 ± 0.03, with a maximum value of 2.43 at sampling site MU04, whereas the mean value for immature individuals was 0.63 ± 0.03, with a maximum of 0.90 at sampling site SE10. Generally, lower values of somatic condition were detected in headwaters for both mature (TUS = 1.28) and immature (SE01 = 0.44) individuals. All population traits at the site level are shown in Table 2.2 and the relationships among population traits are presented in Table 2.3.

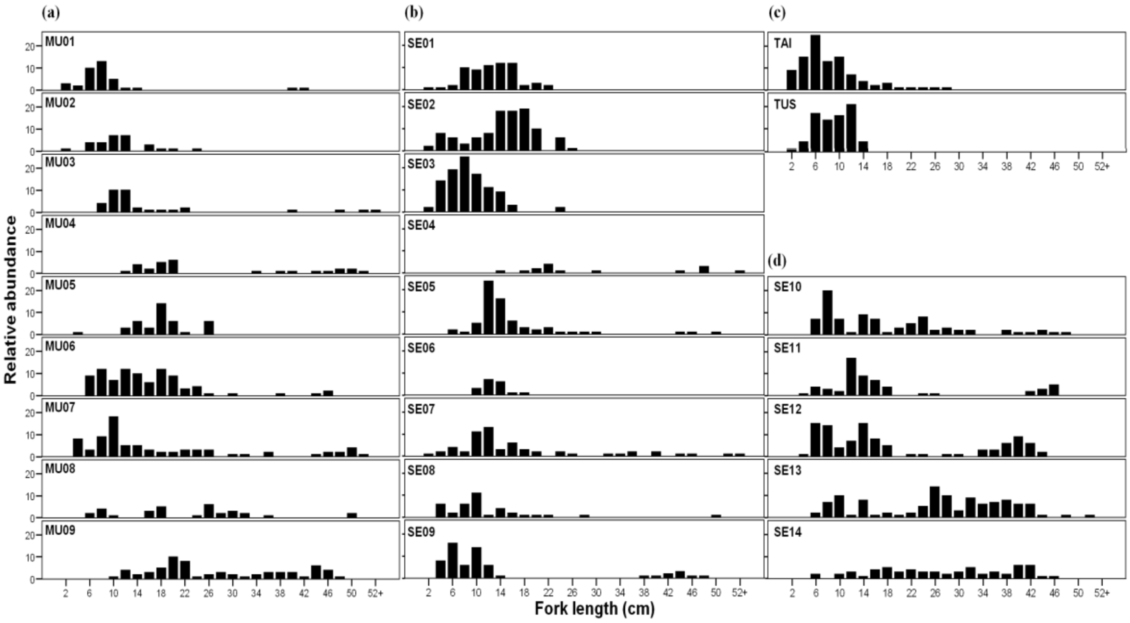

Figure 2.3 Size distribution (2 cm fork length (FL) groups) of *L. sclateri* at sampling site in the Segura River basin (south-east of the Iberian Peninsula). Plot distribution: (a) Mundo River (MU) sampling sites, (b) sampling sites placed from Segura (SE) riverbed to Mundo River confluence, (c) sampling sites at tributaries Tus and Taibilla (TAI) Rivers and (d) sampling sites from Segura–Mundo confluence to the latest downstream sampling site (SE14).

Table 2.2 Population traits of *L. sclateri* at sampling site. N represents the total amount of *L. sclateri* used at each sampling site to determine each parameter. Abundance measured as CPUE (catch per unit of effort); Size parameters as fork length (FL, cm); Age parameters as years; mean value of somatic condition per sampling site as relationship between individual fork length (FL) and total weight (TW); mean growth index as relationship between individual length (FL) and age. All mean values include their confidence level at 95%. This table continues on the next page.

			Size parameters			
Sampling site	n	CPUE	Range	Maximum	Mean (±95% CL)	Diversity index
MU01	37	14.67	40.01	43.81	10.12 ±2.76	1.74
MU02	29	27.43	21.00	25.00	12.21 ±1.72	1.93
MU03	35	19.50	48.50	58.20	17.53 ±4.38	2.00
MU04	28	27.00	41.12	53.72	28.16 ±5.39	2.31
MU05	40	60.00	23.00	27.90	19.11 ±1.49	1.79
MU06	90	90.00	40.61	47.31	16.46 ±1.72	2.43
MU07	78	64.62	48.10	52.20	18.82 ±3.11	2.65
MU08	32	19.40	42.44	50.44	23.59 ±3.97	2.33
MU09	65	79.50	37.11	49.01	28.93 ±2.76	2.77
SE01	65	16.80	19.20	22.20	13.68 ±1.01	2.08
SE02	105	123.60	23.30	27.00	15.47 ±1.05	2.24
SE03	102	111.27	22.40	25.90	10.05 ±0.77	1.94
SE04	15	13.33	38.40	54.40	31.69 ±7.50	2.03
SE05	69	48.00	43.30	50.20	16.80 ±1.91	2.08
SE06	18	19.64	6.70	18.70	14.36 ±0.90	1.35
SE07	60	64.36	50.64	54.44	18.43 ±3.15	2.58
SE08	37	29.45	45.87	51.00	12.81 ±2.81	2.09
SE09	60	78.67	45.35	49.95	14.19 ±3.38	2.03
SE10	85	62.18	43.08	49.91	18.75 ±2.35	2.57
SE11	59	72.00	41.45	47.12	19.29 ±3.25	2.21
SE12	102	88.00	38.78	44.73	20.67 ±2.60	2.55
SE13	111	45.50	48.57	54.97	27.59 ±2.13	2.81
SE14	61	54.00	38.54	46.04	28.53 ±2.79	2.87
TAI	98	150.00	26.10	29.20	9.46 ±1.06	2.15
TUS	77	117.33	11.50	15.20	10.12 ±0.60	1.69
TOTAL	1558	59.85	55.20	58.20	17.77 ±0.56	2.21

Table 2.2 — continued.

	Age parameters				Immature condition		Mature condition	
Sampling site	n	Range	Maximum	Mean (±95% CL)	n	Mean (±95% CL)	n	Mean (±95% CL)
MU01	18	5	5	2.28 ±0.61	11	0.55 ±0.18	26	1.33±0.21
MU02	25	10	10	3.92 ±0.88	2	0.56 ±4.01	27	1.51±0.15
MU03	16	11	14	5.25 ±1.83	0		34	1.84±0.17
MU04	17	10	13	6.59 ±1.84	0		28	2.43±0.23
MU05	33	8	9	5.67 ±0.62	1	0.53	39	2.03±0.08
MU06	37	6	7	3.89 ±0.58	4	0.86 ±0.08	86	1.76±0.11
MU07	28	8	9	4.29 ±0.93	10	0.56 ±0.11	67	1.91±0.17
MU08	27	13	14	6.37 ±1.22	0		31	2.11±0.22
MU09	28	6	9	6.04 ±0.57	0		59	2.40±0.12
SE01	24	8	8	3.92 ±0.82	3	0.44 ±1.03	61	1.57±0.08
SE02	48	9	9	4.35 ±0.72	14	0.55 ±0.12	91	1.84±0.06
SE03	52	7	7	3.10 ±0.44	27	0.61 ±0.08	75	1.34±0.08
SE04	11	10	15	9.27 ±2.62	0		10	2.28±0.15
SE05	31	4	7	4.81 ±0.33	2	0.84 ±0.27	67	1.80±0.10
SE06	13	1	6	5.08 ±0.17	0		18	1.64±0.07
SE07	30	15	15	6.27 ±1.38	7	0.67 ±0.24	53	1.93±0.17
SE08	23	10	11	4.83 ±1.02	8	0.61 ±0.13	29	1.62±0.20
SE09	38	15	15	5.08 ±1.51	13	0.74 ±0.08	36	1.82±0.26
SE10	29	7	9	4.38 ±0.81	2	0.90 ±0.52	82	1.88±0.14
SE11	32	6	8	5.34 ±0.50	3	0.80 ±0.29	56	1.93±0.16
SE12	55	12	14	5.67 ±1.02	10	0.77 ±0.05	92	1.99±0.16
SE13	26	8	8	3.73 ±0.86	1	0.73	108	2.39±0.12
SE14	17	8	9	5.76 ±1.24	47		61	2.42±0.14
TAI	50	10	10	3.38 ±0.69	14	0.57 ±0.08	51	1.48±0.11
TUS	40	5	6	3.65 ±0.38	0	0.65 ±0.11	63	1.28±0.06
TOTAL	748	15	15	4.71 ±0.20	179	0.63 ±0.03	1350	1.87±0.03

Table 2.2 — continued.

Sampling site	Growth index — age 1		Growth index — age 2		Growth index — mature	
	n	Mean (±95% CL)	n	Mean (±95% CL)	n	Mean (±95% CL)
MU01	16	70.04 ±10.60	14	38.25 ±8.12	3	10.35 ±8.75
MU02	24	65.68 ±5.88	24	41.40 ±5.82	12	19.17 ±6.83
MU03	14	67.00 ±7.26	14	38.48 ±5.35	8	13.18 ±10.12
MU04	11	72.28 ±5.70	11	52.92 ±4.25	9	44.13 ±7.12
MU05	21	74.09 ±6.17	16	47.70 ±5.63	20	32.53 ±3.96
MU06	34	70.21 ±3.98	33	45.07 ±3.69	16	27.58 ±4.39
MU07	28	76.28 ±5.39	27	47.08 ±5.35	11	16.22 ±5.13
MU08	16	77.33 ±6.58	15	48.06 ±5.25	10	26.76 ±7.03
MU09	27	80.38 ±5.16	26	53.52 ±4.37	23	31.78 ±3.50
SE01	22	82.46 ±4.54	22	49.09 ±4.18	12	20.30 ±9.06
SE02	38	74.02 ±4.68	37	42.70 ±4.14	25	14.45 ±5.25
SE03	25	55.09 ±4.16	25	32.12 ±3.63	12	17.29 ±7.41
SE04	7	70.32 ±8.81	6	44.93 ±8.55	6	29.06 ±5.79
SE05	31	68.08 ±5.09	30	41.17 ±4.29	15	14.57 ±3.51
SE06	13	70.14 ±5.94	13	41.37 ±6.81	7	13.48 ±9.64
SE07	18	67.33 ±8.01	18	34.92 ±5.81	11	14.46 ±5.12
SE08	20	63.34 ±5.44	20	34.34 ±4.53	13	0.60 ±6.49
SE09	28	62.65 ±3.07	25	31.25 ±2.17	8	2.66 ±4.09
SE10	27	61.40 ±4.68	27	38.30 ±4.60	13	14.77 ±5.81
SE11	30	52.13 ±5.46	30	27.14 ±5.08	23	5.23 ±4.72
SE12	44	69.38 ±3.78	44	41.73 ±3.25	18	17.85 ±5.76
SE13	24	79.10 ±7.12	23	50.72 ±5.05	5	31.36 ±11.36
SE14	17	75.87 ±6.43	16	49.97 ±4.17	13	25.01 ±4.09
TAI	40	50.04 ±3.58	38	25.78 ±2.27	18	4.31 ±4.75
TUS	36	45.72 ±3.82	36	19.26 ±3.59	12	0.03 ±4.90
TOTAL	611	66.74 ±1.28	590	39.34 ±1.13	323	17.69 ±1.57

Table 2.3 Coefficients of the Spearman rank correlation between population traits. Significant differences are indicated: *$p < 0.05$; **$p < 0.001$. Results for rho > 0.7 are marked in bold.

Population traits	Size range	Mean size	Maximum size	Size divers. index	Age range	Mean age	Maximum age	CPUE	S. cond. immature	S. cond. mature	GI — age 1	GI — age 2
Mean size	0.29											
Maximum size	0.87*	0.54*										
Size diversity index	0.44*	0.61*	0.43*									
Age range	0.29	0.14	0.37**	0.11								
Mean age	0.26	0.78*	0.50*	0.33	0.47*							
Maximum age	0.33	0.34	0.50*	0.17	0.92*	0.68*						
CPUE	-0.22	-0.28	-0.39**	0.16	-0.16	-0.30	-0.20					
S. cond. immature	0.52*	0.28	0.54*	0.37	-0.16	0.16	0.03	0.33				
S. cond. mature	0.32	0.97*	0.54*	0.65*	0.26	0.80*	0.43*	-0.22	0.19			
GI — age 1	0.08	0.59*	0.24	0.49*	-0.02	0.32	-0.01	-0.32	-0.39	0.59*		
GI — age 2	-0.01	0.69*	0.22	0.50*	0.01	0.40*	0.05	-0.37**	-0.33	0.69*	0.88*	
GI — mature	-0.14	0.67*	0.15	0.35*	0.00	0.40*	0.06	-0.30	-0.16	0.66*	0.67*	0.88*

2.3.2 Environmental factors

The first three PC axes obtained from the dimension reduction of environmental variables and gradient descriptors explained 79.7% of the total variance (Table 2.4). PC1 was associated with habitat alteration and fragmentation, which were directly related to poor ecological status and low RQI, high values of conductivity and water temperature and low connectivity (high values for ICL and low values of free reach). PC2 was associated with the longitudinal gradient, which was directly related to high altitude, IHF and low water temperatures. PC3 was associated with flow regime alteration, which was directly related to flow variability and MDBF.

Table 2.4 Loadings and proportions of variance extracted by PCA of environmental variables with varimax rotation. Loadings > 0.50 are marked in bold.

Environmental variables	PC1	PC2	PC3
Ecological status	**0.817**	−0.150	0.202
Fluvial Habitat Index (IHF)	0.081	**0.882**	0.036
Riparian Quality Index (RQI)	**−0.648**	0.427	−0.286
Altitude	−0.339	**0.832**	−0.071
Free reach	**−0.909**	0.052	0.087
Longitudinal connectivity (ICL)	**0.869**	0.021	−0.068
Conductivity	**0.750**	−0.443	0.393
Water temperature	**0.520**	**−0.534**	0.466
Mean daily base flow (MDBF)	0.170	−0.473	**0.700**
Flow variability	−0.038	0.164	**0.950**
Proportion of variance	0.366	0.241	0.191

2.3.3 Effects of environmental factors on population traits

Table 2.5 displays the best models obtained from the model selection analysis based on AICc. The longitudinal gradient (PC2) was significantly linked with size-related parameters and somatic condition metrics. The gradient from the upper sampling sites to the downstream sites was associated with increment in population traits including the size range, mean and maximum size, size diversity index and somatic condition metric (longitudinal gradient axis, Figure 2.4). The longitudinal gradient interplayed with other stressors such as the non-native fish species, showing a significant effect on size range and maximum size (size-related parameters, Table 2.5). In addition, this environmental factor interplayed with the flow regime alteration (PC3), displaying a significant effect on mean size (size-related parameters, Table 2.5) and somatic condition for mature individuals (somatic condition, Table 2.5). Finally, the longitudinal gradient also interplayed with habitat alteration and fragmentation (PC1), displaying a significant effect on the size diversity index (size-related parameters, Table 2.5) and somatic condition in immature individuals (somatic condition, Table 2.5).

In addition to the interplay with the longitudinal gradient, the flow regime alteration (PC3) was linked to variability in the age range (age-related parameters, Table 2.5) and the growth index (GI) for mature fish and fish aged 2 years (growth, Table 2.5). An increase in the PC3 axis implied high flow variability and base flow (Impact 2, Figure 2.2c) and was associated with increased mean size and somatic condition of mature individuals, and GI at age 2 years and for mature individuals (flow regime axis, Figure 2.4). In contrast, a decrease in the PC3 axis implied low base flow and variability (Impact 1, Figure 2.2b) and was associated with a decreased age range (flow regime alteration axis, Figure 2.4).

In addition to the interplay with the longitudinal gradient, non-native fish species were linked to the variability in the mean and maximum age (age-related parameters, Table 2.5). An increased relative abundance of non-native fish species was associated with an increase in these two age-related parameters (non-native fish species axis; Figure 2.4).

Finally, habitat alteration and fragmentation (PC1) were linked to the size diversity index (size-related parameters, Table 2.5) and somatic condition of immature individuals (somatic condition, Table 2.5) and always exhibited interplay with the longitudinal gradient. An increase in habitat alteration and fragmentation was associated with increased size diversity index (habitat alteration and fragmentation axis, Figure 2.4). Furthermore, the only interaction found in this study occurred between this stressor and the longitudinal gradient, which was associated with increased somatic condition for immature individuals (longitudinal gradient axis, Figure 2.4). Finally, the model selection analysis did not find any stress factors among those considered in this study to explain the variability in the growth index (GI) of fish at age 1 year or abundance (CPUE).

Table 2.5 General linear models (GLM) results for the best models obtained by model selection analysis based on the Akaike Information Criterion with a correction for small samples sizes (AICc). GLM analysis was conducted for each population trait: CPUE, size- and age-related parameters, growth and somatic condition. (• $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). This table continues on the next page.

SIZE-RELATED PARAMETERS

Size range

Longitudinal gradient + Non-native species

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	26.264	5.239	5.013	5.81e−05	***
Longitudinal gradient	−4.660	1.921	−2.426	0.024	*
Non-native species	18.278	8.792	2.079	0.050	•

Mean size

Longitudinal gradient + Flow regime alteration

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	18.420	1.114	16.537	1.62e−13	***
Longitudinal gradient	−3.097	1.119	−2.768	0.0115	*
Flow regime alteration	2.578	1.117	2.307	0.0313	*

Maximum size

Longitudinal gradient + Non-native species

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	29.456	5.913	4.981	6.27e−05	***
Longitudinal gradient	−4.812	2.168	−2.220	0.038	*
Non-native species	23.839	9.924	2.402	0.026	*

Size diversity index

Longitudinal gradient + Habitat alteration and fragmentation

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	2.231	0.051	43.977	< 2e−16	***
Longitudinal gradient	−0.023	0.051	−4.434	0.0002	***
Habitat alteration and fragmentation	0.117	0.056	2.098	0.048	*

ABUNDANCE (CPUE)

CPUE

Null

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	61.525	7.786	7.902	5.3e−08	***

AGE-RELATED PARAMETERS

Age range

Flow regime alteration

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	8.765	0.519	16.900	4.36e−14	***
Flow regime alteration	−1.586	0.520	−3.048	0.006	**

Table 2.5 — continued.

Mean age Non-native species

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	3.237	0.749	4.324	2.73e−04	***
Non-native species	2.985	1.245	2.397	0.025	*

Maximum age Non-native species

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	7.206	1.658	4.345	2.59e−04	***
Non-native species	5.060	2.759	1.834	0.080	•

SOMATIC CONDITION

Immature somatic condition Longitudinal gradient * Habitat alteration and fragmentation

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	0.642	0.023	28.162	9.97e−14	***
Longitudinal gradient	−0.119	0.028	−4.291	0.0007	***
Habitat alteration and fragmentation	−0.015	0.028	−0.552	0.589	
L. gradient : H. alter. and fragment.	0.110	0.043	2.578	0.022	*

Mature somatic condition Longitudinal gradient + Flow regime alteration

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	1.869	0.058	32.211	< 2e−16	***
Longitudinal gradient	−0.188	0.058	−3.228	0.004	**
Flow regime alteration	0.107	0.058	1.844	0.080	•

GROWTH

Growth index (GI) at age 1 Null

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	66.676	2.387	27.930	< 2e−16	***

Growth index (GI) at age 2 Flow regime alteration

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	40.724	1.723	23.639	<2e−16	***
Flow regime alteration	3.607	1.728	2.087	0.049	*

Growth index (GI) for mature Flow regime alteration

	Estimate	Std. Error	t value	Pr(>|t|)*p*	
(Intercept)	18.165	2.042	8.894	9.73e−09	***
Flow regime alteration	5.614	2.049	2.740	0.012	*

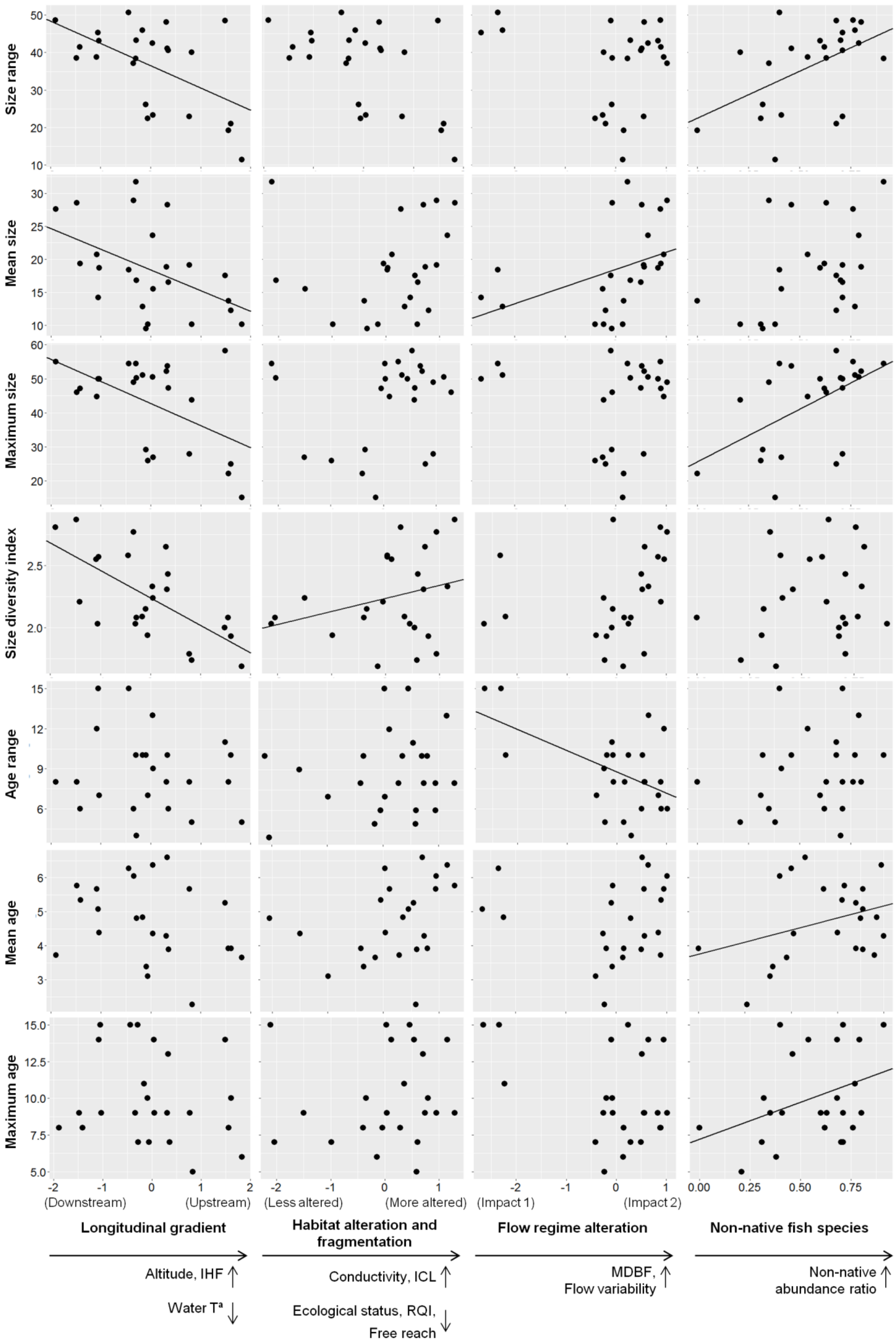

Figure 2.4 Relationship between environmental factors (longitudinal gradient, habitat alteration and fragmentation, flow regime alteration and non-native fish species) and *L. sclateri* population traits at sampling site in the Segura River basin (south-east of the Iberian Peninsula). Significant results (GLM $p < 0.05$) are represented with marked trend line on the plot. In x-axes, arrows pointing down indicate decrease in environmental variables (i.e., water temperature), and arrows pointing up indicate increase in environmental variables (i.e., altitude) and also increase in non-native abundance ratio. This figure continues on the next page.

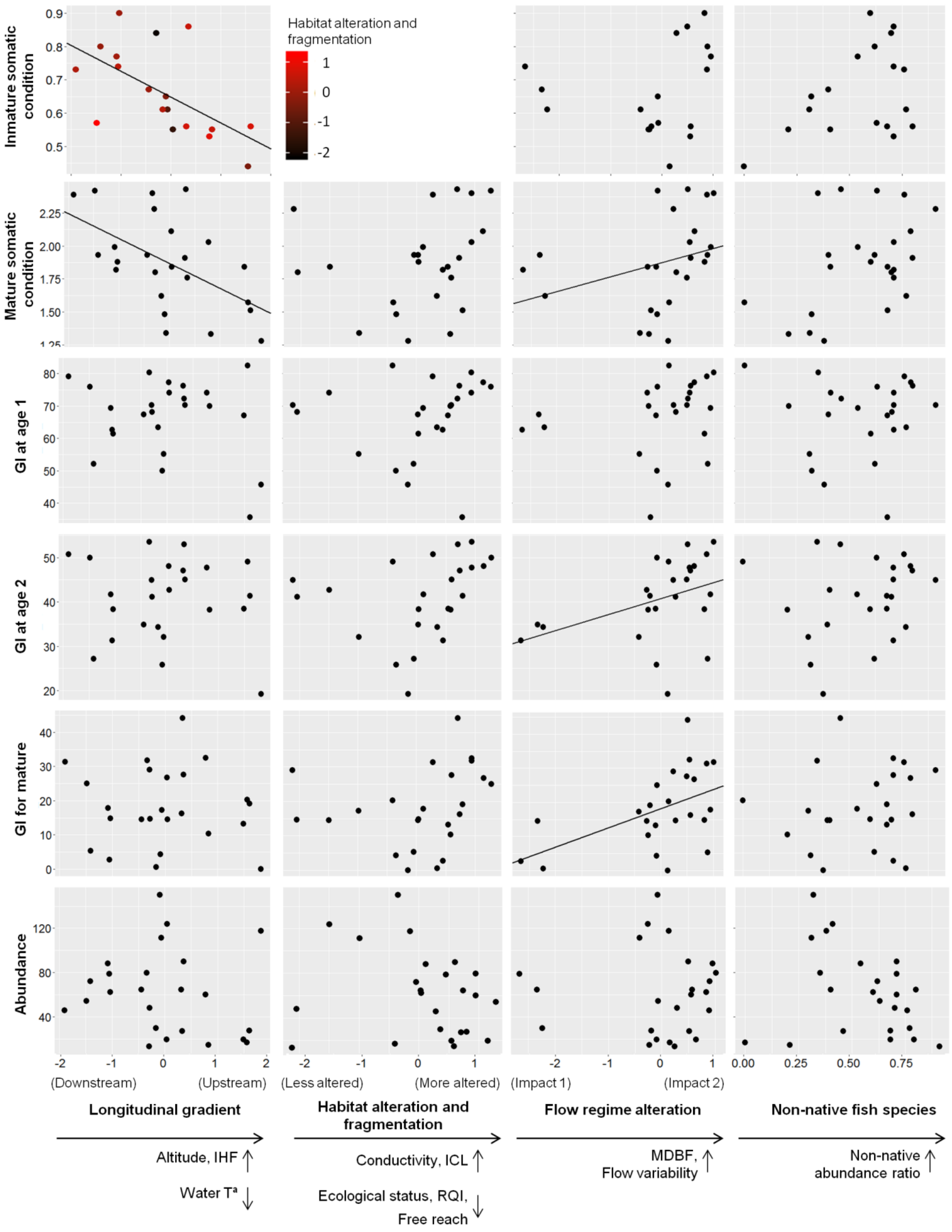

Figure 2.4 — continued.

2.4 Discussion

In this study, we analyzed the variation in population traits of *L. sclateri* in response to environmental factors in the fluvial reaches of the Segura River basin. Our results confirm significant variability in population traits along the longitudinal gradient due to the effect of human impacts, mostly those related to flow regulation such as fragmentation and habitat alteration, flow regime alteration and the relative abundance of non-native fish species. The use of biological traits related to size and age, relative abundance, growth and somatic condition allowed us to identify the population-level responses of this sentinel Iberian fish to different stressors and to verify the complex effect of multiple stressors in a heavily modified Mediterranean-type river.

The longitudinal gradient is a key factor in the structure and dynamic of freshwater ecosystems, so it is essential to consider its effects in the assessment of multiple stressors in river systems (Hermoso et al., 2009; Magalhães et al., 2002; Pringle, 2001; Radinger et al., 2018). Environmental conditions along the longitudinal gradient determine the availability of resources such as food, refuges and breeding areas (Belmar et al., 2013; Mas-Martí et al., 2010). Consequently, a marked effect of the spatial variation on fish populations was expected, especially in Mediterranean-type rivers which exhibit a strong climatic and altitudinal gradient (Gasith and Resh, 1999; Hughes et al., 2010; Wootton, 1998). The increment of resources downstream along natural river systems promotes large sizes and a wider range of size classes in the fish fauna (Alexandre et al., 2015; Magalhães et al., 2002; Mas-Martí et al., 2010). We found significant variability in the size parameters and somatic condition of *L. sclateri* populations associated with spatial variation, with an increase in these biological traits along the longitudinal gradient. The size population structure displayed a polymodal pattern, with high variability among sampling sites. The size distribution results highlighted a lack of some size groups, and this was more evident in sampling sites downstream of reservoirs. Small and medium-sized individuals were scarce downstream of operational dams (i.e., at sites MU09, SE04, SE09 and SE11). The GLM results (Table 2.5) showed that size and somatic condition parameters were significantly associated with the longitudinal gradient. In fact, habitat alteration and fragmentation, flow regime alteration and relative abundance of non-native fish species were all related to the variability of population parameters, reflecting the severe alterations that have affected the Segura River basin (Belmar et al., 2013; Grindlay et al., 2011).

Human impacts increase along the longitudinal gradient as a consequence of the greater accessibility to water resources (Hughes et al., 2010). As a result, the interplay among spatial variation and human stress factors shaped the environmental conditions that act as a “filter” of biological traits (Poff et al., 1997). The selected traits determine the biological responses of freshwater fauna to cope with altered conditions (Bunn and Arthington, 2002; Hermoso et al., 2009; Poff et al., 2010). The Segura River basin shows a strong influence of human alterations mainly related to agricultural supply (Bruno et al., 2014; Grindlay et al., 2011). Agricultural practices involve strong hydraulic management that results in a loss of connectivity (habitat fragmentation), flow regime alteration and water quality and habitat degradation and encourages the establishment of non-native fish species (Bunn and Arthington, 2002; Clavero et al., 2013; Ribeiro et al., 2008; Sánchez-Pérez et al., 2020). Our results show the interplay between environmental stress factors, mostly those related to flow regulation, and the longitudinal gradient in some biological traits such as size and somatic condition. In addition, we found that age and growth variations were significantly associated with the isolated effect of the flow regime alteration and the relative abundance of non-native fish species. This finding suggests that the magnitude of human impacts in the study area could be masking some ecological responses to longitudinal gradients (Hermoso et al., 2011; Tedesco et al., 2009).

The flow regime is considered the main driver of freshwater ecosystems, defining the structure, function and dynamic of rivers, and affecting the individual fitness and growth rate of fish populations (Lytle, 2001; Mims and Olden, 2012; Poff et al., 1997). We found significant relationships between flow regime alteration and some population traits of *L. sclateri* including mean size, age range, somatic condition for mature individuals and growth variability. Our results show an increase in these population traits associated with fluvial sectors that had a high level of base flow throughout the year (reflected as Impact 2 in Figure 2.2c) and a flow regime pattern that reduced the strong seasonal variability of Mediterranean-type rivers. Although these fluvial sectors display an inversion in flow seasonality related to agricultural water demands, they also provide an increased availability of water, refuge and food resources

(Alexandre et al., 2014a; Mas-Martí et al., 2010; Vila-Gispert and Moreno-Amich, 2001). Furthermore, high flow levels promote changes in body shape and muscle development, which induce better swimming performance and increased somatic condition (Alexandre et al., 2014b; Langerhans, 2008), a finding that was previously documented in the Segura River basin (Oliva-Paterna et al., 2003a). In contrast, we observed a decrease in population traits (mean size, age range, somatic condition for matures and growth variability) associated with strong inversion in flow seasonality and base flow reduction (Impact 1 in Figure 2.2b). We found this flow pattern in sampling sites downstream of the Cenajo reservoir, a consequence of the operating characteristics of its dam (Amat-Trigo, 2018; Belmar et al., 2013; Piqué et al., 2016). The extreme hydrological conditions caused by this type of water regulation result in a poor-quality habitat, especially for adult barbels (Boavida et al., 2015), and are considered a limiting factor for the growth of barbel species in the Iberian Peninsula (Alexandre et al., 2014a; Oliveira et al., 2002). These two contrasting results in the response of fish populations under different hydrological flow patterns have been described in other Iberian rivers as well (Alexandre et al., 2014a; Merciai et al., 2017b).

Non-native fish species tend to be dominant in human-altered ecosystems, such as the Mediterranean rivers, where more stable environmental conditions that result from flow regulation measures encourage their establishment and spread (Clavero et al., 2013; Ribeiro et al., 2008). Iberian fish communities have exhibited significant changes over recent decades as a consequence of the introduction of a wide range of non-native fish species (Clavero and García-Berthou, 2006; Leunda, 2010; Martínez-Fernández et al., 2019), and these changes are especially evident in the Segura River basin (Oliva-Paterna et al., 2014). The negative responses of native fish populations associated with the presence and abundance of non-native species in the fish assemblage are well documented (Cucherousset and Olden, 2011; García-Berthou et al., 2015; Hermoso et al., 2009). Our results show an increase in the maximum size and size range, and the mean and maximum age of *L. sclateri* populations associated with a higher ratio of non-native fish abundance. The proliferation of non-native species is generally a result of changes in the environmental conditions caused by flow regulation (Maceda-Veiga, 2013; Vörösmarty et al., 2000). The lack of small size classes of fish at sampling sites placed downstream of reservoirs where the presence of non-natives is favoured (i.e., SE04 in Figure 2.3) suggests an effect of the fish assemblage composition on the structure of *L. sclateri* populations. In general, predation by non-native fish could affect the population structures and dynamics of native fish species in the Iberian Peninsula (Leunda, 2010). Some studies from other Iberian rivers confirmed the inclusion of different barbel species in the diet of top predator fish such as *E. lucius* (Domínguez and Pena, 2001; Rincón et al., 1990) and *S. Lucioperca* (Pérez-Bote and Roso, 2012). Predatory fish (*E. lucious, S. lucioperca* and *M. salmoides*) showed a higher occurrence in the lower reaches of the study area. They could be inducing higher predation pressure on certain size classes of *L. sclateri* and therefore affecting the population size structure as Bravo et al. (2001) showed in the Palancar River, where *M. salmoides* predation was directly related to the lack of 0+ individuals of dominant species such as *L. sclateri.*

The results of this study highlight relevant associations between human impacts, most of which were related to flow regulation, and the population traits of *L. sclateri* along a longitudinal gradient, providing insights into the population-level responses of this sentinel Iberian fish to environmental conditions at the site level. The key role of the longitudinal gradient in driving the increase in human impacts as a result of greater accessibility related to the lower reaches of rivers is evident, since most of the stressors were related to the spatial variation, so this dependence makes it difficult to interpret the effect of isolated stressors. In addition, there is a wide variety of human impacts present in the study area that were not considered in this study. For example, pollution could be driving the response of *L. sclateri* to environmental conditions (Sánchez-Pérez et al., 2020), or predatory mammals (*Lutra lutra*), whose predation on *L. sclateri* in the Segura River basin was recently confirmed (Dettori et al., 2021).

The ability of this species to adapt to changes in local conditions has been shown by the variation in population size/structure along the longitudinal gradient. Although human impacts exerted significant effects on the biological traits we evaluated, our findings also suggest that the wide inter-population plasticity displayed by *L. sclateri* may be a mechanism for this species to successfully inhabit a highly modified Mediterranean-type river. Cyprinids in general show great adaptability to environmental alterations (Alexandre et al., 2014a; Encina et al., 2006; Oliveira et al., 2002; Ruiz-Navarro et al., 2016), and *L. sclateri* showed a tolerance to the effects of flow regulation previously studied in the same river basin (Oliva-Paterna et al., 2003b; Torralva et al., 1997).

In recent decades, declines in fish populations have been documented for several Iberian fish species and there have been drastic reductions in fish species that were previously widely distributed in the study area. Native fish species are vulnerable to the rapid increase in human pressure on the water resource; this is especially so in Mediterranean areas where an increase in the magnitude of extreme weather events is expected under climate change scenarios (IPCC, 2019). Therefore, the use of well-known and widely distributed sentinel species, such as *L. sclateri*, may prove a useful tool to increase the knowledge of the adaptability and population responses to gradients of single and multiple stressors, which is essential to establish and improve management actions to protect native fish species.

Chapter 3

Multi-species fishways in a Mediterranean-type river: contributions as migration corridors and compensatory habitat for cyprinids

Abstract

River connectivity is essential for the resilience of fish assemblages and populations, and is a priority goal to reach good ecological status for river systems. Increasing knowledge on the functionality of restoration tools such as fishways is relevant for future management strategies. The present two-year assessment showed clear ecological contributions of different types of multi-species fishways in the fish assemblage of a strongly modified Mediterranean-type river. Just after their implementation, early and extended use by dominant river-resident fish of both nature-like and technical fishways were observed. All fishways were used as migration corridors, especially during the movement period by potamodromous cyprinids. Fishways also provided key compensatory habitats for small and juvenile individuals throughout the annual cycles, mostly for rheophilic fish inside nature-like bypasses and for limnophilics inside technical types. Fluvial habitat characteristics and lower flow variability inside the fishways could favour their role as a fish refuge, mainly to juveniles of cyprinids, in heavily regulated rivers where large flow fluctuations occurred. Nature-like fishways seem to be the most appropriate to restore connectivity for rheophilic cyprinids in Mediterranean-type rivers, even more because their use by large non-native limnophilics seems to be very scarce. Therefore, nature-like designs should be considered more intensively in restoration planning. However, technical fishways could offer the opportunity to establish control traps of some non-native fish, which could be of interest to reduce the risk of spreading invasive fish. The obtained information on the ecological functionality of multi-species fishways should be considered for applying successful river restorations that are demanded by water and wildlife management schemes (e.g., the European Water Framework Directive).

3.1 Introduction

The loss of connectivity due to anthropogenic barriers within river systems is considered to be among the main emerging threats and a leading cause of the global decline in freshwater biodiversity (Reid et al., 2018). These artificial barriers are built to meet human needs such as increased energy demand or the expansion of irrigated agriculture, causing severe hydrological and geomorphologic modifications (Dudgeon et al., 2006; Olden et al., 2010; Vitousek et al., 1997), with almost half of the global freshwater volume already altered by flow regulation (Grill et al., 2019). Fluvial connectivity is essential for river dynamics and for the resilience of freshwater biota (Pringle, 2003); thus, artificial obstacles that alter river hydrology and generate discontinuities in key environmental variables (e.g., water temperature or substrate composition) could potentially induce changes in freshwater community composition and structure (Jones et al., 2020; Mueller et al., 2011). Fish fauna are specially affected by artificial barriers such as dams and weirs that promote habitat fragmentation and severely affect their population connectivity and migration (Branco et al., 2017; Jones et al., 2021; Radinger et al., 2017; Silva et al., 2017).

Human development in Europe has caused massive changes in rivers for centuries, making European rivers probably the most heavily fragmented by artificial barriers in the world (Belletti et al., 2020) with the Mediterranean areas being especially affected (Hermoso et al., 2017; Hooke, 2006; Radinger et al., 2018; Sabater et al., 2018). For instance, more than 50,000 obstacles (large dams and other barriers) have been built since 1960 in Spain, considered one of the most regulated regions in the world, which implies a severe threat to its freshwater biodiversity (Rincón et al. 2017; Brink et al. 2016; Maceda-Veiga 2013; Hermoso and Clavero 2011). Actually, mitigating the effects of river fragmentation is a great challenge for the European Commission with relevant conservation implications and is a priority goal to reach the good ecological status for river systems required by the EU's Water Framework Directive (European Commission, 2000).

Reducing the effects of rivers fragmentation is essential to restore freshwater ecosystem functioning (Pringle, 2003), but the implementation of generalized solutions is difficult due to the specific characteristics of each river, its particular fish community and the effect of each obstacle on the complex dynamics of river systems, which requires a regional or even local approach for successful management (Nilsson et al. 2005; Kemp and O'Hanley, 2010; Terêncio et al. 2021). To mitigate the negative impact of non-passable barriers on fish populations, fishways have been implemented as an effective restoration tool when barrier removal is not feasible (Hermoso et al., 2017; Katopodis and Williams, 2012; Silva et al., 2017). In the past, efforts in the design of fishways have generally focused on species with high socioeconomic relevance, particularly salmonids, with limited success for other non-commercial fish species (Mallen-Cooper and Brand, 2007; Noonan et al., 2012; Ovidio et al., 2017; Roscoe and Hinch, 2010). However, in the last decade, a more holistic approach has implemented new designs for a wider range of species to maintain the whole fish community (Steffensen et al., 2013; Thiem et al., 2012; Tummers et al., 2016). These multi-species fishways have not only functioned as migration corridors, but have also been used as new compensatory habitats for both migratory and resident fish (Pander et al., 2013).

Despite the fact that most fish species have low economic interest, the implementation of fishways is currently focused on maintaining the entire fish community, since these engineering solutions contribute significantly to restoring population connectivity and fish migration (Kemp and O'Hanley, 2010; O'Hanley et al., 2013; Tummers et al., 2016). Taking into account the wide diversity of species with different biological requirements and strategies to deal with passage facilities, successful fishway designs and implementations requires a multidisciplinary working group that involves experts in ecohydraulics, fish behaviour, policy and socioeconomics (Silva et al., 2017). However, just as important as the design process is evaluating the effectiveness of fishways once they are operative (Odeh, 1999; Roscoe and Hinch, 2010). In fact, monitoring programmes are essential to understand the response of fish assemblages to passage facilities, allowing us to maximize their performance and improve future fishway designs (Cooke and Hinch, 2013; Kemp, 2012). Furthermore, the selection of the typology to implement depends on various requirements (e.g. logistical limitations or economic resources) (DVWK, 2002; Travade and Porcher, 2002), and evaluating the use of different fishway typologies under similar environmental conditions allows comparisons and helps in the design of future successful implementations. However, despite the worldwide increase in the construction of multi-species fishways, studies concerning the use and effectiveness of the entire fish assemblage are scarce in the literature (Bunt et al., 2012; Noonan et al., 2012; Pander et al., 2013; Roscoe and Hinch, 2010). The lack of these kinds of studies is also evident in Mediterranean rivers, such as those from the Iberian Peninsula, where the scarcity of fish species with commercial interest limits funding to assess the effectiveness of implemented fishways (Noonan et al. 2012). Most Iberian studies have focused on providing robust information on target species, mainly endemic cyprinids (see Hershey (2021), which positions the Iberian barbel as the fifth most studied species), addressing fish passage to improve fishway designs (e.g., Alexandre et al. (2013b); Branco et al. (2013); Bravo-Córdoba et al. (2021); Romão et al. (2017 and 2019)). The use and effectiveness of fish passages for whole fish assemblages has rarely been studied (Aparicio et al., 2012; Ordeix, 2017). Comprehensive field evaluations remain scarce, and most of them are limited to the reproductive periods of some target species (Sanz-Ronda et al., 2019, 2016; Silva et al., 2015). In sum, basic ecological knowledge underpinning the need for effective fish passages within a fish assemblage approach (e.g., temporal patterns of fish passage use) remains largely scarce in the literature (Silva et al., 2017).

In addition, considering the response of non-native fish to multi-species fishways in highly invaded aquatic ecosystems such as heavily regulated Iberian rivers (Clavero et al., 2013; Fornaroli et al., 2020; Ilhéu et al., 2014; Sánchez-Pérez et al., 2020), could provide insightful information for fish management (Radinger and García-Berthou, 2020; Santos et al., 2012). Since the main objective of the implementation of multi-species fishways is to facilitate movement for a wide range of species, these infrastructures could also facilitate the dispersion of previously contained invasive species (Stuart et al. 2006; McLaughlin et al. 2013; Terêncio et al. 2021). At this point, management decisions could involve conflicts between recovering native species movements and limiting non-native fish spread (Ordeix, 2017; Rahel and McLaughlin, 2018; Starrs et al., 2015). Although management decisions must be implemented locally because the effects of reconnecting isolated stretches could depend on the context of each obstacle (Mclaughlin et al., 2013), understanding how non-native species deal with fishways helps to implement appropriate cost-effective restoration measures (Brevé et al., 2014; Roscoe and Hinch, 2010; Silva et al., 2017).

This study was developed in context of the fish-based monitoring of an extensive river restoration programme, the LIFE+ Segura-Riverlink LIFE12ENV/ES/001140 project (Oliva-Paterna et al., 2016). We aimed to validate the implementation of multi-species fishways as an effective tool to restore fish population connectivity. We investigated the use of five fishways from different typologies (nature-like and technical) by the fish assemblage in a middle section of a Mediterranean-type river (Segura River, south-east of Spain). Seasonally and with a fishway-type approach, we assessed the role of fishways as movement corridors and as compensatory habitats for the dominant river-resident fish with contrasting sizes, swimming capacity, ecological guilds (rheophilic and limnophilic) and behaviour (potamodromous and non-migratory). Based on their ecological traits, it was expected that the fishways would be especially used by dominant cyprinid species during their movement period as migration corridors, although the availability of new habitats inside fishways could expand their use by other species of the fish assemblage. The results may be relevant for future management strategies and help decision-makers implement appropriate restoration measures in highly impacted rivers, since considering the regional features of Mediterranean river systems will allow us to achieve the objectives proposed by the EU's Water Framework Directive.

3.2 Material and methods

3.2.1 Study area and fishways

This study was conducted in the Segura River basin, which is located in the southeastern Iberian Peninsula, a semi-arid region characterized by warm summer temperatures (mean annual temperature = 18 °C) and scarce rainfall (300 mm/year approx.) (Figure 3.1). This Mediterranean basin drains an area of 18,870 km^2 and is distinguished by a marked climatic gradient and severe annual and interannual variations in the natural flow regime, from extreme droughts to torrential floods (Hooke, 2006; Miró et al., 2018). Agricultural practices account for 80% of the total water demands and involve the construction of a large number of different types of flow regulation structures (Aldaya et al., 2019; Bruno et al., 2014). Overall, free water flow is constrained by a total of 33 dams (> 10 m height and > 1 hm^3 of reservoir capacity) and 170 smaller obstacles along the longitudinal gradient. This basin accounts for approximately 1200 hm^3 of storage capacity, and additional water resources (approx. 350 hm^3 year^{-1}) are supplied from interbasin water transfer from the Tagus Basin (Grindlay et al., 2011). This strong modification of the natural flow regime, operating in combination with the pollution derived from agricultural and urban activities (CHS, 2015), causes a severe degradation of riverine habitats (Belmar et al., 2013; Bruno et al., 2014). Consequently, the Segura River basin is considered one of the most human-impacted Mediterranean basins, with a high level of flow regulation (Aldaya et al., 2019; Grindlay et al., 2011).

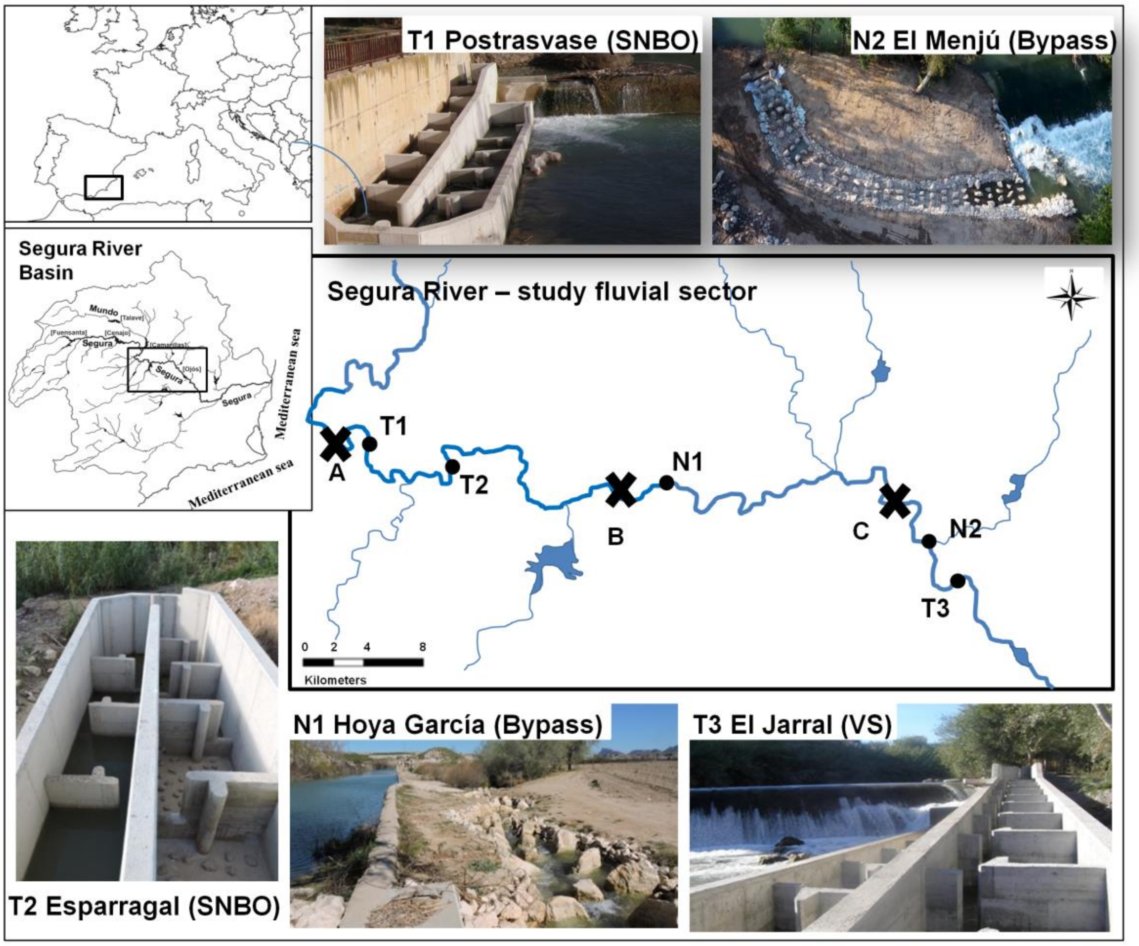

Figure 3.1 Location of sampling sites (●) and gauging/weather stations (A Bayo, B Almadenes and C Menjú) in the Segura River basin and its location in the south-east of the Iberian Peninsula. (N) nature-like fishways; (T) technical fishways: (SNBO) submerged notch with bottom orifice type and (VS) vertical slot type.

This study was carried out at five sampling sites distributed along a fluvial sector 54 km long in the middle part of the Segura River basin (Figure 3.1). The most abundant fish are represented by four cyprinids: the limnophilic *Alburnus alburnus* (Linnaeus, 1758), which is exotic and has invaded large areas in the Iberian Peninsula, and three rheophilic species; the single native fish in the study area *Luciobarbus sclateri* Günther, 1868; and two non-natives: *Pseudochondrostoma polylepis* (Steindachner, 1864) and *Gobio lozanoi* Doadrio & Madeira, 2004. Other exotic and limnophilic fish are locally present, such as *Gambusia holbrooki* (Agassiz, 1859), *Cyprinus carpio*

Linnaeus, 1758 and *Lepomis gibbosus* (Linnaeus, 1758), and some alien piscivore fish occur at low densities (i.e., *Micropterus salmoides* (Lacépède, 1802), *Sander lucioperca* (Linnaeus, 1758) and *Esox lucius* Linnaeus, 1758. Non-native fish species represent 90% of the total richness of the fish assemblage in the studied fluvial sector (Oliva-Paterna et al., 2019, 2014).

Each sampling site included a multi-species fishway and its reach just downstream (100 m long) of the river (Figure 3.1). All the fishways were designed and built in the context of an extensive river restoration programme following standard guidelines (DVWK, 2002; Travade and Porcher) and taking into account hydraulic recommendations for cyprinids (Sanz-Ronda et al., 2019). They started to operate in autumn 2015 (see Oliva-Paterna et al. (2016)), and the fish assemblage use of different types of fishways was evaluated over two annual cycles since January 2016. Two nature-like fishways of rocky bypass type (N1 Hoya García and N2 El Menjú) and three technical stepped fishways, two of them of SNBO type (Submerged Notch with Bottom Orifice; T1 Post-trasvase and T2 Esparragal) and one of VS type (Vertical Slot; T3 El Jarral), were monitored (Figure 3.1; Table 3.1). Bypass types were nature-oriented constructions with variabilities in current speed, water depth and natural bottoms originally composed of rocks. Substrates of both natural and technical fishways were quickly naturalized through sandy and muddy materials carried by the river over a few months of fishway operation. Flow discharge inside the fishways was individually determined (Table 3.1) and regulated by a sluice gate placed in the flow entrance. More descriptive variables of the five monitored fishways and the downstream reaches sampled are presented in Table 3.1.

Table 3.1 Sampling site descriptive variables for nature-like (N1, N2) and technical (T1, T2, T3) fishways; SNBO is a submerged notch with bottom orifice type and VS is a vertical-slot type. Location (UTM30 ETRS 89) with coordinates is provided as Universal Transverse Mercator. Downstream reach just below each fishway included the presence of a pool directly connecting river and fishway.

Sampling site	Name	Location	Fishway characteristics						Downstream characteristics		
			Typology	Slope (%)	Discharge design ($m^3\ s^{-1}$)	Length (m)	Inner area (m^2)	Fishway structure	Mean reach width (m)	Mean water depth (m)	Fishway entrance
N1	Hoya García	X: 627272, Y: 4233623	Bypass	4.0	0.67	51.5	155	Channel with a middle section resting pool	3	0.40	Small or medium pool
N2	El Menjú	X: 639119, Y: 4231252	Bypass	3.5	0.90	52.0	154	Channel with two resting pools	8	1	Small or medium pool
T1	Post-trasvase	X: 613788, Y: 4235661	SNBO	7.3	0.31	34.7	45	11 pools	5	1.20	Small or medium pool
T2	Esparragal	X: 613787, Y: 4235662	SNBO	7.7	0.35	43.9	55	15 pools	3	0.20	Small or medium pool
T3	El Jarral	X: 640577, Y: 4229308	VS	6.5	0.29	43.8	61	17 pools	14	1.20	Large pool >5 m^2

3.2.2 Sampling design and fishway monitoring

Between 26 and 28 sampling events inside each fishway were conducted during two complete annual cycles (from January 2016 to December 2017), with the beginning of the sampling period being approximately three months from the operation start date. The passage use by the whole fish assemblage was quantitatively assessed. However, we selected the four dominant cyprinids in the fluvial sector (*L. sclateri, P. polylepis*, *G. lozanoi* and *A. alburnus*) as target fish for the assessment of the ecological contributions (i.e., migration corridors and compensation habitats) of the newly built fishways. Moreover, according to their ecology, we defined the movement period as the time window encompassed jointly by spring and summer months (Froese and Pauly, 2021; Oliva-Paterna et al., 2019).

At least two sampling events inside fishways per season were carried out during autumn and winter, but during the defined target fish movement period, each fishway was sampled twice a month. Furthermore, seasonal qualitative samplings were conducted in downstream reaches (a 100-m-long river stretch) placed just downstream of each fishway.

Previously to conduct the sampling, both the fishway entrance and exit were blocked by nets (0.5 cm mesh size) to avoid the escape of the fish inside the fishway. Afterwards, the flow discharge was notably reduced by closing the sluice gate of the fishways, thus allowing the catchability of potentially all individuals inside the pass (total captures) by electro-fishing. Two electro-fishing passes (1800 W generator, working voltage between 200 and 350 V, 2–3 A) were consecutively conducted by the same surveyor team for each sampling event, following the CEN standard protocol (CEN, 2003). Individuals were stunned by a single surveyor holding an anode, whereas two other surveyors collected stunned fish with dip nets and transferred them to plastic containers supplied with oxygen. All specimens using the fishways at that time were captured, as ensured from the two electro-fishing passes. Downstream reaches were previously sampled by electro-fishing passes (30-35 min) in wadable 100-m-long sections (qualitative sampling), which were also blocked by nets to avoid fish escape. All captured fish were anaesthetized using clove oil (50 mg/l diluted in ethanol in a 1:10 proportion), subsequently identified to the species level, counted and measured (fork length FL; ±1 mm).

For the assessment of fish passability directions and to complement information on passage use, mark-recapture data were evaluated at three sampling sites: N2 El Menjú (bypass), T2 Esparragal (SNBO) and T3 El Jarral (VS). Individuals larger than 8 cm of fork length (FL) belonging to the four target species were marked with Visible Implant Elastomers (VIE-tags) using different colour codes for captures inside the fishways and at downstream reaches. Recaptured individuals were re-marked in a different part of the body using a specific combination of VIE-tags that allowed us to differentiate (1) how many times each fish was caught and (2) whether it was previously marked inside the fishway or at a downstream reach.

Individuals were manipulated in accordance with the European Union Directive 2010/63/UE on animal welfare in scientific activities and Spanish Act RD 53/2013, following the ethical guidelines of Murcia University and with the approval of the competent authorities (Regional Government on Natural Resources and Water Management Authority), under authorization AUF20150077. Moreover, significant efforts were made to minimize stress and the mortality rate was less than 1%. Once the fish were recovered from the anaesthesia, all fish were released at the sampling site.

3.2.3 Environmental variables

On a fortnightly basis, river discharge (m^3 s^{-1}) and air temperature (°C) were analyzed for the studied period using online databases provided by the official monitoring service of the Segura Hydrographic Confederation at three gauging or weather stations distributed along the study area (Figure 3.1) (https://www.chsegura.es/chs/cuenca/redesdecontrol/estadisticashidrologicas/; accessed 22 January 2018). Following the methodology applied by Fuentes-Pérez et al. (2017), the flow discharge (m^3 s^{-1}) inside each fishway was calculated at each sampling event. In addition, water temperature was measured at each sampling site, with three replicates both inside fishways and in their respective downstream reaches, using a multiparameter (Multi 340i WTW).

3.2.4 Statistical analysis

The Lilliefors test (Kolmogorov–Smirnov) was used to test the normality of the captured data inside each fishway. Then, a non-parametric analysis of variance (Kruskal–Wallis test) was applied to test differences among capture rates of target species inside each fishway. Differences between species occurrence and size distribution during the movement or migratory period (spring and summer) and the non-movement period (autumn and winter) for the target fish inside each fishway were tested by the Wilcoxon test.

Spearman rank correlation analysis was used to test the correlations between environmental variables, and a p value < 0.05 was considered significantly correlated (rho coefficient > 0.7 was applied to establish a strong correlation between variables).

We used general linear models (GLMs) to test the effects of environmental variables (flow discharges, water and air temperatures) on the captures inside fishways (Clarke et al., 2014). This analysis was performed with the *glm* function in the R package *car*. The sampling period (movement and non-movement period) and sampling site (each fishway) were included as factors to test the influence of different movement periods and types of fishways. GLMs were conducted using the total number of captures and the captures of each target species. All statistical analyses were performed using R software.

3.3 Results

3.3.1 Fish occurrence and captures

A total of ten fish species were detected in the study area during the electrofishing surveys, most of which were present in the downstream reaches of the five sampling sites, with the exception of *E. lucius*, which was only present in T1 and T2, and *G. hoolbroki*, which was only detected in N1, N2 and T3 (Table 3.2). T2 and T3 showed the highest species richness inside fishways, with eight out of nine species present in their respective sampling sites. Five species were detected inside all the fishways: *L. sclateri, P. polylepis*, *G. lozanoi, A. alburnus* and *L. gibbosus.* In contrast, two species were only detected inside one fishway (*E. lucius* inside T2 and *G. hoolbroki* inside N1). The four largest non-native fish (*C. carpio, M. salmoides*, *S. lucioperca* and *E. lucius*) were not detected inside the nature-like fishways N1 and N2 (Table 3.2).

Table 3.2 Occurrence of fish species and total richness recorded at each sampling site (N1, N2, T1, T2 and T3) monitored in the main stem of the Segura River (south-east of the Iberian Peninsula). Data are presented for each sampling site and divided into: inside fishways (I) and at downstream reaches (D).

	N1		N2		T1		T2		T3	
Fish species	I	D	I	D	I	D	I	D	I	D
A. alburnus	X	X	X	X	X	X	X	X	X	X
C. carpio	-	X	-	X	-	X	-	X	X	X
E. lucius	-	-	-	-	-	X	X	X	-	-
G. hoolbroki	X	X	-	X	-	-	-	-	-	X
G. lozanoi	X	X	X	X	X	X	X	X	X	X
L. gibbosus	X	X	X	X	X	X	X	X	X	X
L. sclateri	X	X	X	X	X	X	X	X	X	X
M. salmoides	-	X	-	X	X	X	X	X	X	X
P. polylepis	X	X	X	X	X	X	X	X	X	X
S. lucioperca	-	X	-	X	-	X	X	X	X	X
Species richness	6	9	5	9	6	9	8	9	8	9

A total of 12,772 fish were caught inside the five fishways from January 2016 to December 2017, with the four target fish accounting for 98.8% of the total captures (*A. alburnus* 50.0%, *G. lozanoi* 37.5%, *L. sclateri* 4.3% and *P. polylepis* 7.1%). A total of 61.1% of the total fish captured inside the fishways occurred inside T3 (VS type), followed by nature-like fishways, which provided 15.7% of the total captures (N2), and 10.7% (N1). Conversely, the SNBO type had the lowest values, with 7.6% (T2) and 5.0% (T1) of the total captures.

Alburnus alburnus and *G. lozanoi* jointly represented 86.5% (48.2% *A. alburnus* and 38.3% *G. lozanoi*) of the total fish captured inside the fishways. Both were the most representative species inside each fishway (Figure 3.2), accounting for >70% of the total captures inside each fishway with the exception of N2. *G. lozanoi* was the dominant species inside all the fishways (except inside T3), followed by *A. alburnus* (except inside N2). For both species, the Kruskal–Wallis test revealed that species captures differed among fishways: *G. lozanoi* ($\chi^2_4 = 28.505$, $p < 0.001$) and *A. alburnus* ($\chi^2_4 = 27.309$, $p < 0.001$). Pairwise Wilcoxon post hoc comparisons revealed that mean captures of *G. lozanoi* showed differences between T3 and both SNBO types (T1 and T2) and between T1 and N2. At the same time, *A. alburnus* captures only were significantly different inside T3, where a single sampling event yielded 2,212 *A. alburnus* captured, which represented 17.3% of the total individuals of this species captured inside T3. This fishway accounted for the highest captures of both species: 85.4% of the total *A. alburnus* captured and 44.2% of the total *G. lozanoi* (Figure 3.3). In the case of *A. alburnus*, the results of a single sampling event markedly skewed its total captures, as mentioned previously.

P. polylepis and *L. sclateri* accounted jointly for 12.2% (7.1% and 5.1%, respectively) of the total fish captured inside the fishways (Figure 3.2). The Kruskal–Wallis test also revealed that the mean captures of both species differed among sampling sites: *P. polylepis* ($\chi^2_4 = 19.618$, $p < 0.001$) and *L. sclateri* ($\chi^2_4 = 17.131$, $p = 0.001$). Pairwise Wilcoxon post hoc comparisons revealed that the mean captures of both species were significantly higher inside N2, which accounted for 60.0% and 47.3% of the total captures of *P. polylepis* and *L. sclateri*, respectively (Figure 3.3).

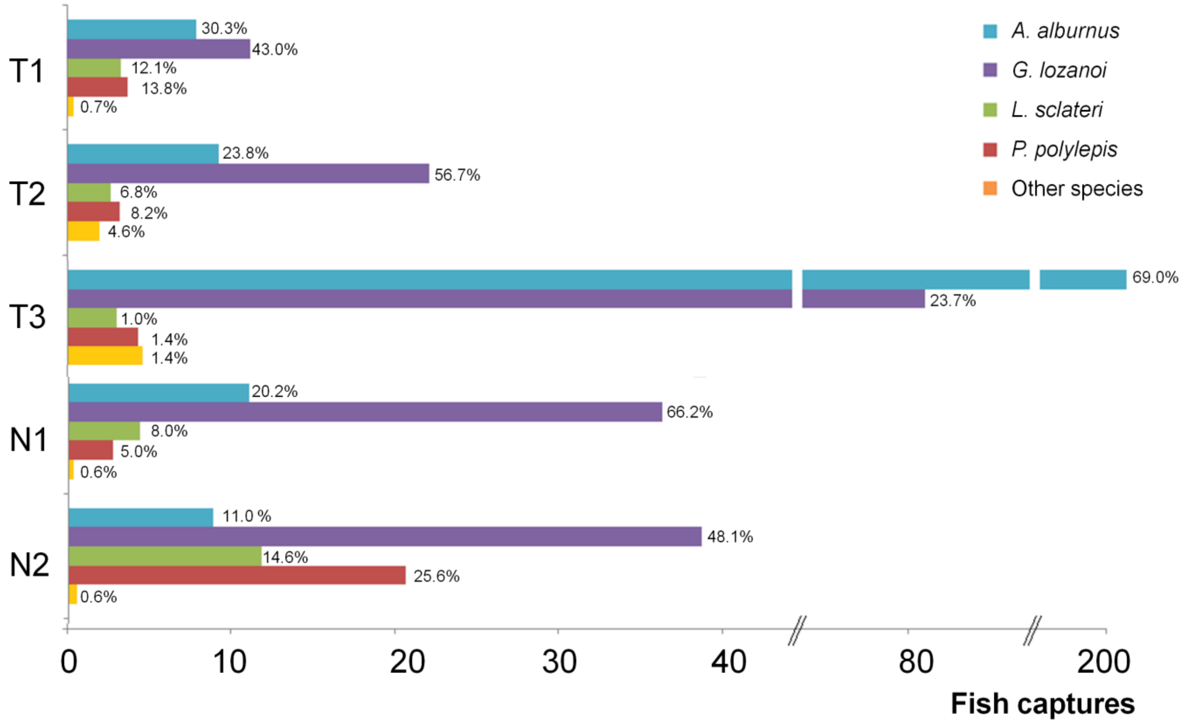

Figure 3.2 Frequency distribution of fish captures inside the studied fishways. Data are shown as average captures per sampling event during the study period and percentage of each target fish species and non-target grouped as “other species” per fishway. The sampling sites include technical fishways (T1, T2 and T3) and nature-like fishways (N1 and N2).

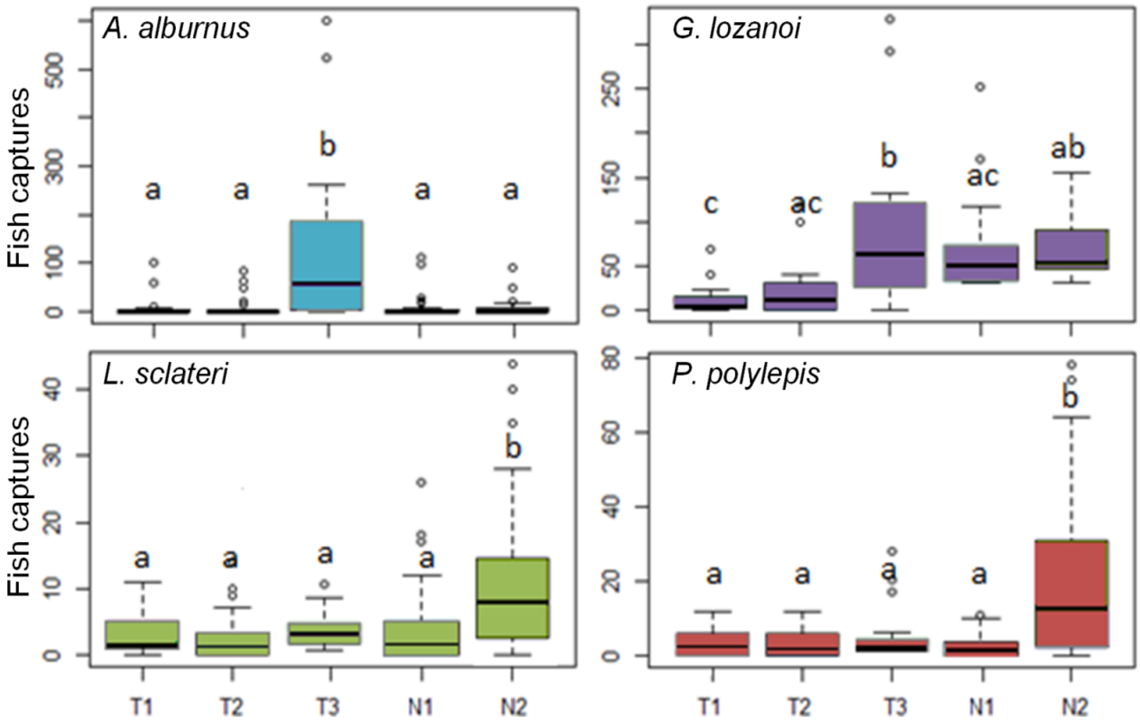

Figure 3.3 Boxplots comparing total of captures during the study period for the target species inside each of the studied fishways (T1, T2, T3, N1 and N2). Mean (central line), 25th and 75th percentile values, as well as maximum and minimum are represented in boxplot. Letters above boxes indicate groupings based on significant differences ($p < 0.05$) obtained from pairwise Wilcoxon tests.

Significant differences (Wilcoxon post hoc tests) are shown by groups (letters) on boxplots (Figure 3.3), and the results are summarized in Table 3.3.

Table 3.3 Results of pairwise post-hoc Wilcoxon comparisons for captures of each target species between fishways (N1, N2, T1, T2 and T3). Post-hoc Wilcoxon test ($p < 0.05$).

		N1	N2	T1	T2
A. alburnus	N2	1.000			
	T1	1.000	1.000		
	T2	1.000	1.000	1.000	
	T3	0.0007	0.00104	0.00052	0.00037
G. lozanoi	N2	0.5780			
	T1	0.1566	0.0017		
	T2	0.5780	0.1566	0.5780	
	T3	0.0595	0.1156	0.0002	0.0018
L. sclateri	N2	0.0384			
	T1	1.000	0.0219		
	T2	1.000	0.0071	1.000	
	T3	1.000	0.0148	1.000	1.000
P. polylepis	N2	0.0047			
	T1	1.000	0.0180		
	T2	1.000	0.0064	1.000	
	T3	1.000	0.0111	1.000	1.000

3.3.2 Temporal use of fishways

Fish richness and total captures inside fishways showed temporal variations over the study period. There were no sampling events with any fish caught inside N2 and T3 or in any downstream reaches. Only four sampling events (3% of the total number of samplings), mostly at the beginning of the studied period, showed no captures inside fishways (two inside T1 (February 2016 and March 2017), one inside T2 (March 2016) and one inside N1 (March 2016)). During the entire study period, the four target species showed higher occurrences than the remaining fish species, which were generally detected inside fishways in less than 7.0% of the sampling events. The rheophilics *L. sclateri, G. lozanoi* and *P. polylepis* generally showed higher occurrences during the movement period, being detected in more than 70% of the total sampling events, while the occurrences decreased by approximately 15.0% for *G. lozanoi* and *P. polylepis* and 26.2% for *L. sclateri* during the non-movement period. In fact, some of these species were also captured significantly more often inside technical fishways (mainly the SNBO type) during the movement period (Wilcoxon test results; Figure 3.4). In contrast, the occurrence of limnophilic *A. alburnus* inside the fishways was more temporally stable, being detected in 38.7% of the sampling events during the movement period and in 51.4% sampling events during the non-movement period, with the exception of T3, where this species showed more than 90% occurrence in both periods (Figure 3.4).

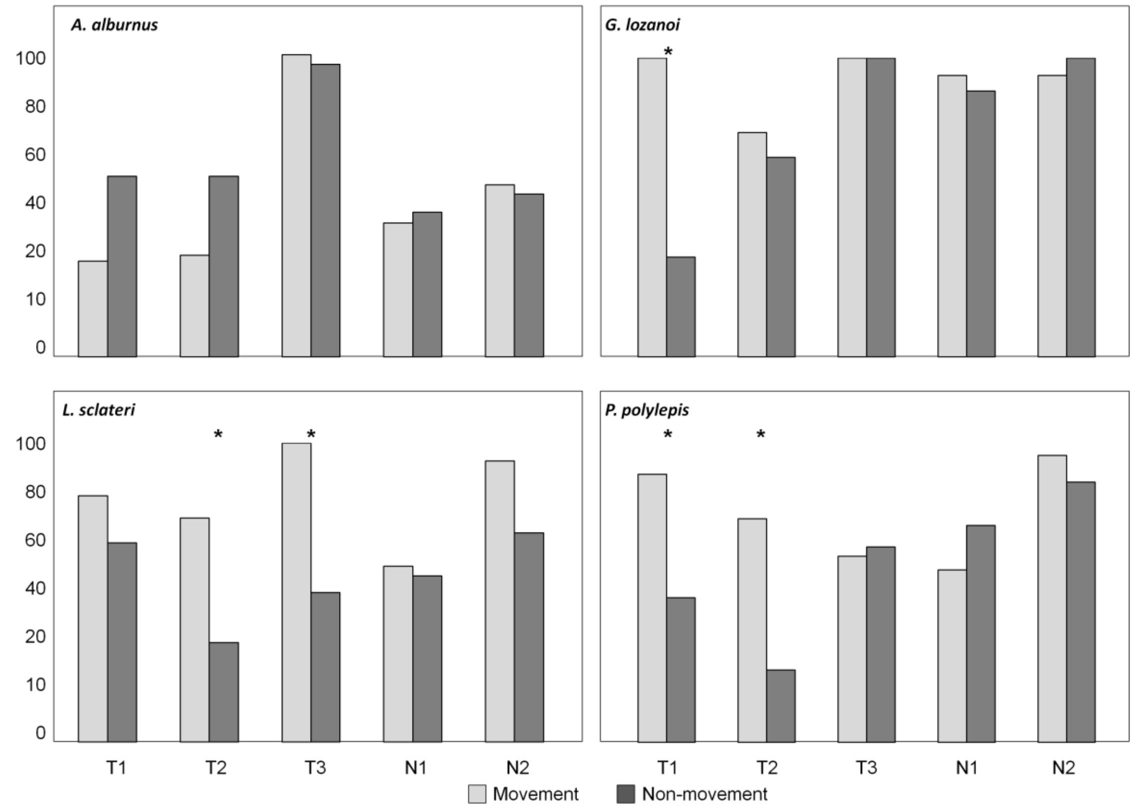

Figure 3.4 Frequency of occurrence of the target species during the movement (light gray bars) and the non-movement (dark grey bars) periods inside the fishways (T1, T2, T3, N1 and N2) monitored. Significant differences between-periods obtained from Wilcoxon test ($p < 0.05$) are indicated with asterisks above bars.

The temporal patterns of total captures per site of the target species are presented in Figure 3.5. Winter captures were generally scarce, except inside N2, which accounted for 31.9% of captures for 2017. Fish were also scarce during the first spring season in which fishways were operating (2016), when captures were lower than 10% of the annual captures inside each of the fishways (ranging from 9.8% (T1) to 3.1% (T3)), but increased to an average of 26.3% in spring 2017 (from 46.4% (T1) to 16.4% (T2)). Sixty percent of the total captures of the four target species occurred during the non-movement period, but these proportions varied between species and fishways.

In contrast to the remaining target species, the captures of *L. sclateri* inside the fishways were mostly concentrated during the movement period (70.6%, ranging from 84.4% (T2) to 61.9% (N1)). Its greatest captures occurred during summer but were also relatively high in autumn inside nature-like fishways (Figure 3.5). In contrast, *A. alburnus* showed most of its captures during the non-movement period inside all the fishways (66.6%, ranging from 81.7% (T2) to 50.3% (N2)). This non-native species usually showed peaks in autumn (as highlighted by an extraordinary event of 2,212 individuals captured inside T3 on 26th November), although captures were also relatively high during summer inside technical fishways. The captures of *G. lozanoi* and *P. polylepis* were higher during the non-movement period inside nature-like fishways and inside T3 but were conversely higher during the movement period inside the SNBO type (T1 and T2). Neither species showed marked capture peaks and displayed relatively constant abundance during the study period inside N2 or inside T3 in the case of *G. lozanoi* (Figure 3.5).

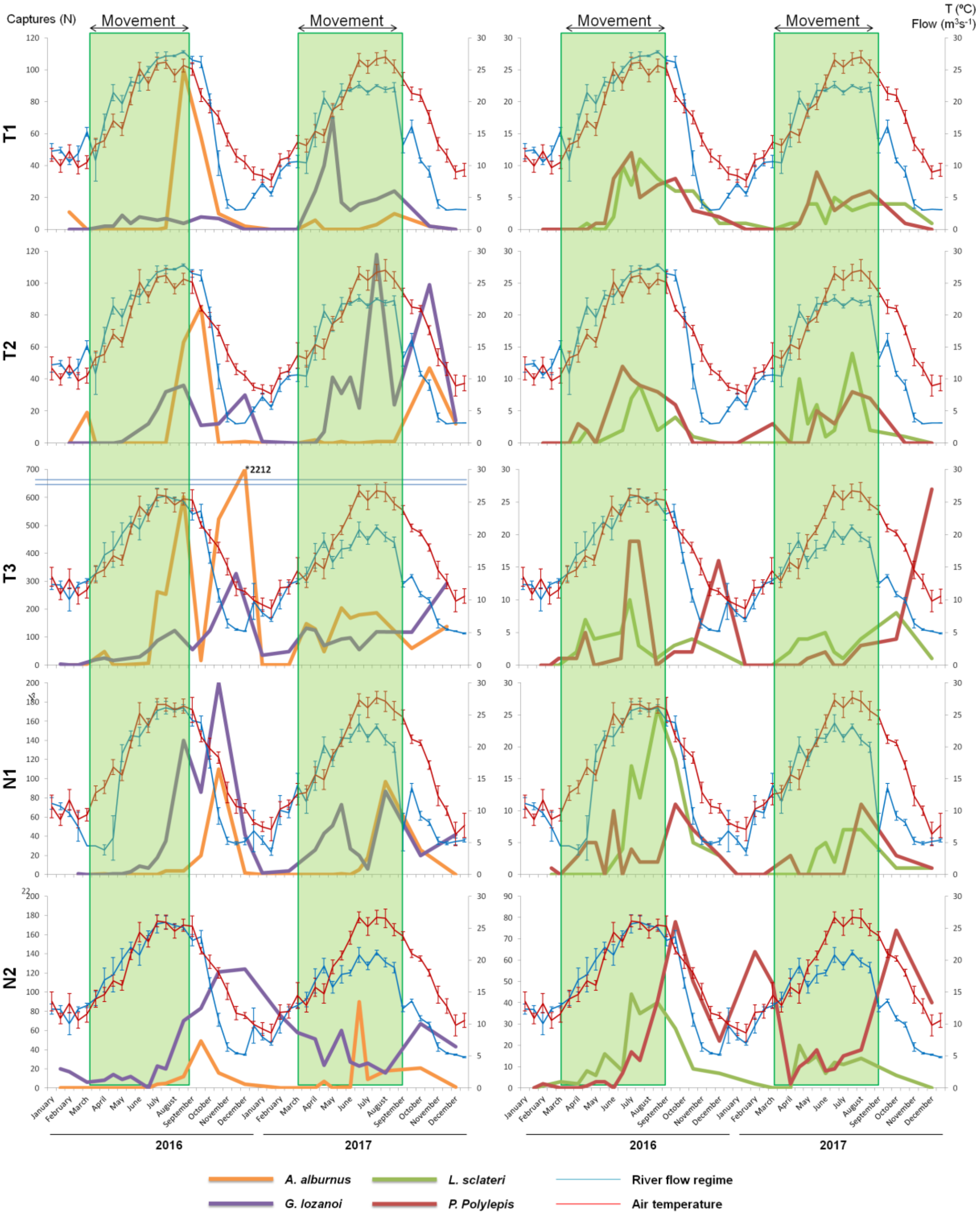

Figure 3.5 Temporal variation in captures for the four target fish species during the study period inside the fishways (T1, T2, T3, N1 and N2) monitored in the main stem of the Segura River (south-east of the Iberian Peninsula). Fortnightly values of river flow discharge and air temperature were also represented for the study period and movement periods were highlighted.

Luciobarbus sclateri did not show significant differences in size (FL) between the movement and non-movement periods inside any fishway (Wilcoxon test, $p > 0.05$), although larger individuals (more than 300 mm FL) were more abundant or were exclusively found during the movement period (Figure 3.6). Other rheophilic species, such as *G. lozanoi,* displayed significantly larger sizes during the movement period inside all the fishways with the exception of T1. This pattern was also shown by *P. polylepis* and *A. alburnus* inside N1 and N2. In sum, nature-like fishways promoted the use of large individuals of the target species during the movement period. In contrast, technical fishways displayed smaller sizes during the movement period in some cases, such as *A. alburnus* inside T2 or T3 and *P. polylepis* in T3 (Figure 3.6).

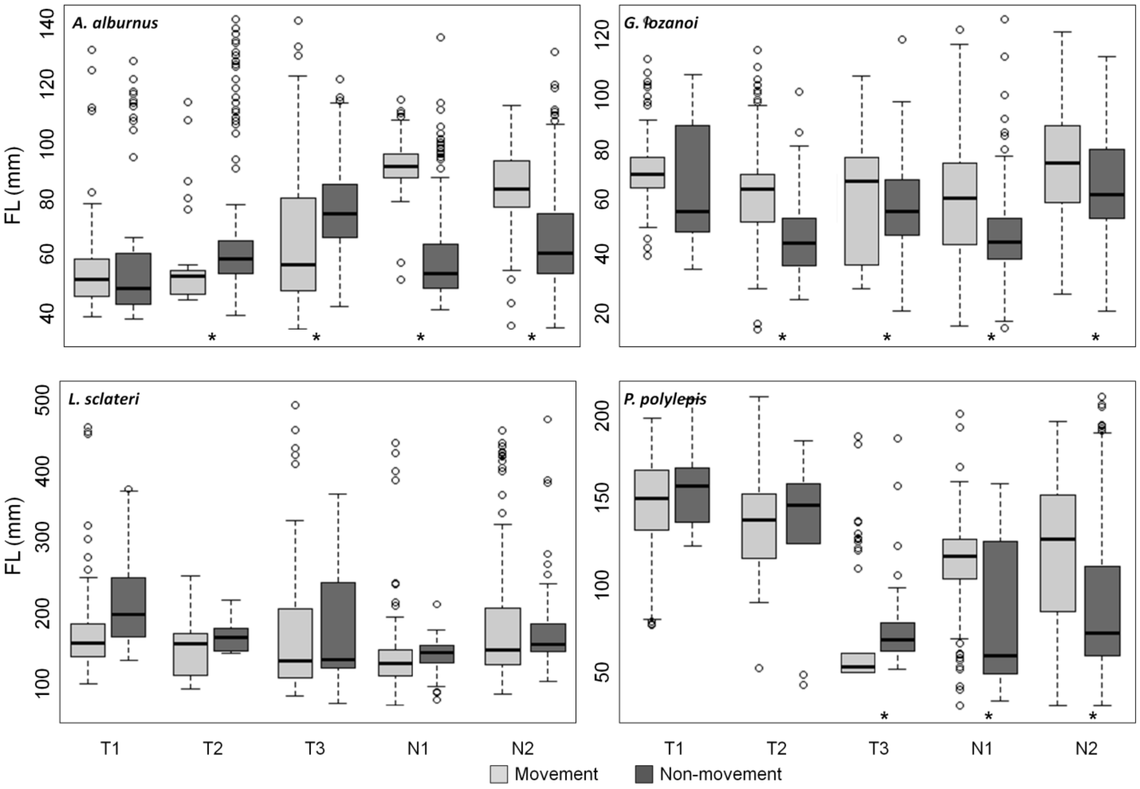

Figure 3.6 Boxplots comparing fish size (FL: fork length in mm) for the target species during the movement and the non-movement period inside the fishways (T1, T2, T3, N1 and N2). Boxplots display mean (central line), 25th and 75th percentile, as well as maximum and minimum with outliers. Significant differences in fish size between periods are indicated with asterisks (Wilcoxon test, $p < 0.05$).

3.3.3 Mark-recapture data

A total of 2,002 individuals of target species were marked at the three selected sampling sites (T2, T3 and N2): 1075 inside the fishways (763, 154 and 185 inside each fishway) and 927 in downstream reaches (109, 679 and 139 in each site). A total of 18.3% of the fish marked within the fishways were later recaptured while making use of these passage facilities; in addition, 5.5% were recaptured twice, and 0.3% were recaptured up to three times. The N2 fishway had the highest proportions of recaptures: 20.2% for first recaptures; 7.1% for seconds and 0.4% for third recaptures, and was the only fishway with one individual captured four times (Table 3.4).

In downstream reaches, 5.7% of marked fish were recaptured, and only 0.1% was recaptured twice. The downstream reach in sampling site T2 showed the greatest proportion of recaptures, with 7.2% for first recaptures, and was the only downstream reach with second recaptures (Table 3.4). *L. sclateri* was the species most marked; accounting for 37.8% of the fish marked inside the fishways and 52.3% in downstream reaches. This species was also the most recaptured, comprising 31.5% of the recaptured individuals marked inside the fishways and 8.5% in downstream reaches. Moreover, *G. lozanoi* and *P. polylepis* showed high recapture values inside the fishways, 24.4% and 17.3%, respectively. Finally, *A. alburnus* accounted for only 2.7% of the individuals marked inside the fishways. Fish passability (= upstream movement to enter inside the fishway or downstream movement to leave the fishway) was confirmed by recaptures at two sampling sites (T2 and N2) in concordance with the detection of specimens with different colour codes in VIE-tags. In general, the fish passability ratios (percentage of fish passability) of marked fish were relatively low: 0.85% for upstream and 0.68% for downstream movements. Up- and downstream movements were shown for both *L. sclateri* and *G. lozanoi*, whereas *P. polylepis* only displayed upstream passage. No fish passability was detected for *A. alburnus*, probably due to the small number of tagged fish.

Table 3.4 Fish-passability ("yes" in up and down movements) for the target fish species at the three sampling sites (T2, T3 and N2) where the mark-recapture survey was conducted. Percentage of the total recaptures inside each fishway an in their respective downstream reaches are shown.

	T2	T3	N2
Up-movement	Yes	-	Yes
Down-movement	-	-	Yes
Recaptures inside			
1st recapture	14.94%	13.51%	20.24%
L. sclateri	56.5%	48.8%	47.7%
P. polylepis	43.5%	-	24.8%
A. alburnus	-	4.0%	-
G. lozanoi	-	48.0%	27.5%
2nd recapture	2.60%	1.62%	7.06%
L. sclateri	100%	33.3%	51.99%
P. polylepis	-	-	30.8%
G. lozanoi	-	66,70%	30,8
3rd recapture	-	-	0.41%
G. lozanoi	-	-	33.3%
P. polylepis	-	-	66.7%
Downstream recaptures			
1st recapture	7.22%	1.44%	1.83%
L. sclateri	77.6%	50%	100%
P. polylepis	16.3%	50%	-
G. lozanoi	6.1%	-	-
2nd recapture	0.15%	-	-
L. sclateri	100%	-	-

3.3.4 Environmental variables

Air temperature showed no differences throughout the study area because significant correlations were found between the data obtained from the three weather stations (Spearman $p < 0.05$). In addition, a strong correlation (Spearman $rho > 0.7$) was found between the air and water temperatures at each sampling site (Table 3.5, Figure 3.7). In the case of water temperature, during the study period, it ranged from 8.2 to 24.8 °C, with values higher than 20 °C from late May to early October.

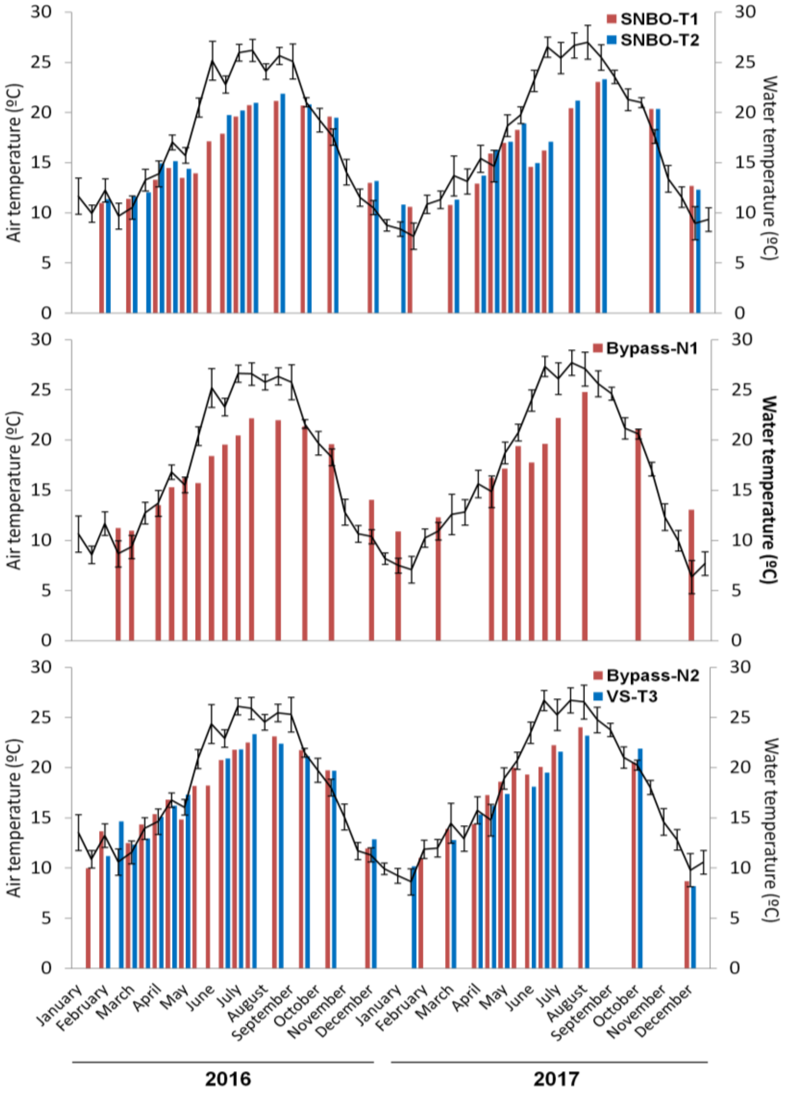

Figure 3.7 Temporal air and water temperature variation (ºC) during the study period in the Segura River (south-east of the Iberian Peninsula). Air temperature was obtained from three weather stations (A, B and C in Figure 3.1). Water temperature was measured in-situ at sampling date in each sampling site (N1, N2, T1, T2 and T3).

River flow discharge did not show differences throughout the study area, and significant correlations (Spearman $p < 0.05$) were found between the river flows discharges obtained from the three gauging stations. However, the results did not display strong correlations between river flow discharge and flow discharge inside the fishways (Table 3.5), which is expected according to the hydrological design of fishways to maintain stable flow discharges. The flow regime in the study area is characterized by a strong inversion of the natural pattern, showing high flows during spring and summer months and low flows during autumn and winter (Amat-Trigo et al., 2021; Sánchez-Pérez et al., 2020). River flow discharge ranged from 13.8 to 27.8 m^3s^{-1}, with values higher than 20 m^3s^{-1} from May to September.

Table 3.5 Correlations between environmental variables at each sampling site ((N1, N2, T1, T2 and T3). Data of river flow discharge and air temperature obtained from three gauging/weather stations: for T1 and T2 from A, for N1 from B and for N2 and T3 from C in Figure 3.1. Strong Spearman rank correlation was established by *rho* value (> 0.7) and was marked in bold (** $p < 0.05$; *$p < 0.1$).

		River flow discharge	Flow inside fishway	Air tempterature
T1	Flow inside fishway	-0.30		
	Air tempterature	**0.84****	-0.33	
	Water temperature	0.66**	-0.26	**0.78****
T2	Flow inside fishway	0.70**		
	Air tempterature	**0.85****	0.70**	
	Water temperature	**0.73****	**0.73****	**0.85****
T3	Flow inside fishway	0.56**		
	Air tempterature	**0.80****	0.64**	
	Water temperature	**0.74****	0.61**	**0.91****
N1	Flow inside fishway	0.34*		
	Air tempterature	**0.83****	0.29	
	Water temperature	**0.71****	0.36*	**0.88****
N2	Flow inside fishway	0.67**		
	Air tempterature	**0.81****	0.59**	
	Water temperature	**0.73****	0.63**	**0.92****

The potential effects of environmental variables (river flow discharge, flow inside the fishway and water temperature) on the captures inside fishways were studied using GLMs (see Table 3.6 and Table 3.7). Water temperature and sampling site (fishway) were significantly related to the total captures inside the fishways with no interaction of the sampling period. Only in *P. polylepis* the results highlighted a significant effect of the sampling period. This result is related to the significant use of the fishways that the target fish make during the whole annual cycle and could reflect a similar effect of the temperature in any period of the year, with the exception of *P. polylepis*. Total captures inside fishways showed a significant increase with water temperature (Figure 3.8), with T3 being the most differentiated fishway with a significant interaction (estimate = 9.91, $p < 0.001$). For *G. lozanoi* and *A. alburnus*, we found an interaction between the sampling site and water temperature, with T3 being the site significantly affected (*G. lozanoi*: estimate = 5.98, $p = 0.017$; *A. alburnus*: estimate = 9.92, $p < 0.001$).

Table 3.6 General linear models (GLM) conducted for assessing effects of environmental variables on total fish captures and on captures of each target fish species inside fishways (dependent variables). Only significant effects of environmental variables or factors were shown (*$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Six outliers identified as data above the 95th percentile were not included in the models.

Total of captures	χ^2	Df	Pr ($>\chi^2$)	
Sampling site	36.94	1	$1.21\text{x}10^{-08}$	***
Water temperature	46.93	4	$1.58\text{x}10^{-07}$	***
Sampling site:Water temperature	14.25	4	0.0065	**
L. sclateri				
River flow discharge	9.14	1	0.0025	**
Water temperature	21.49	1	$3.56\text{x}10^{-05}$	***
Sampling site	69.88	4	$2.41\text{x}10^{-13}$	***
River flow discharge:Water temp:Sampling site	25.63	4	$3.77\text{x}10^{-04}$	***
P. polylepis				
Water temperature	23.46	1	$1.28\text{x}10^{-05}$	***
Sampling site	75.09	4	$1.91\text{x}10^{-14}$	***
Movement period	19.04	1	$1.28\text{x}10^{-04}$	***
Sampling site:Movement period	57.29	4	$1.08\text{x}10^{-11}$	***
G. lozanoi				
Water temperature	16.38	1	$5.18\text{x}10^{-04}$	***
Sampling site	31.25	4	$2.72\text{x}10^{-05}$	***
Sampling site:Water temperature	14.60	4	0.0056	**
A. albunus				
Water temperature	25.47	1	$4.50\text{x}10^{-06}$	***
Sampling site	63.45	4	$5.47\text{x}10^{-13}$	***
Sampling site:Water temperature	21.41	4	$2.63\text{x}10^{-04}$	***

Table 3.7 General linear model (GLM) conducted for assessing effects of environmental variables on total fish captures inside each fishways (dependent variables; T1, T2, T3, N1, N2). Significant effects of environmental variables or factors were shown (*$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Six outliers identified as data above the 95th percentile were not included in the models.

T1	χ^2	Df	Pr (>χ^2)	
Flow inside fishway	0.57	1	0.4501	
River flow discharge	2.48	1	0.1151	
Air temperature	2.00	1	0.1569	
Water temperature	6.66	1	0.0099	**
Movement period	<0.01	1	0.9977	
T2				
Flow inside fishway	<0.01	1	0.9760	
River flow discharge	0.35	1	0.5536	
Air temperature	1.00	1	0.3169	
Water temperature	8.81	1	0.0030	**
Movement period	0.79	1	0.3729	
T3				
Flow inside fishway	4.11	1	0.0426	*
River flow discharge	5.32	1	0.0211	*
Air temperature	0.07	1	0.7883	
Water temperature	0.65	1	0.4208	
Movement period	0.18	1	0.6748	
N1				
Flow inside fishway	<0.01	1	0.9760	
River flow discharge	0.35	1	0.5536	
Air temperature	1.00	1	0.3169	
Water temperature	8.81	1	0.0030	**
Movement period	0.79	1	0.3729	
N2				
Flow inside fishway	3.37	1	0.06627	•
River flow discharge	0.01	1	0.9283	
Air temperature	0.17	1	0.6841	
Water temperature	6.34	1	0.0118	*
Movement period	8.88	1	0.0029	**

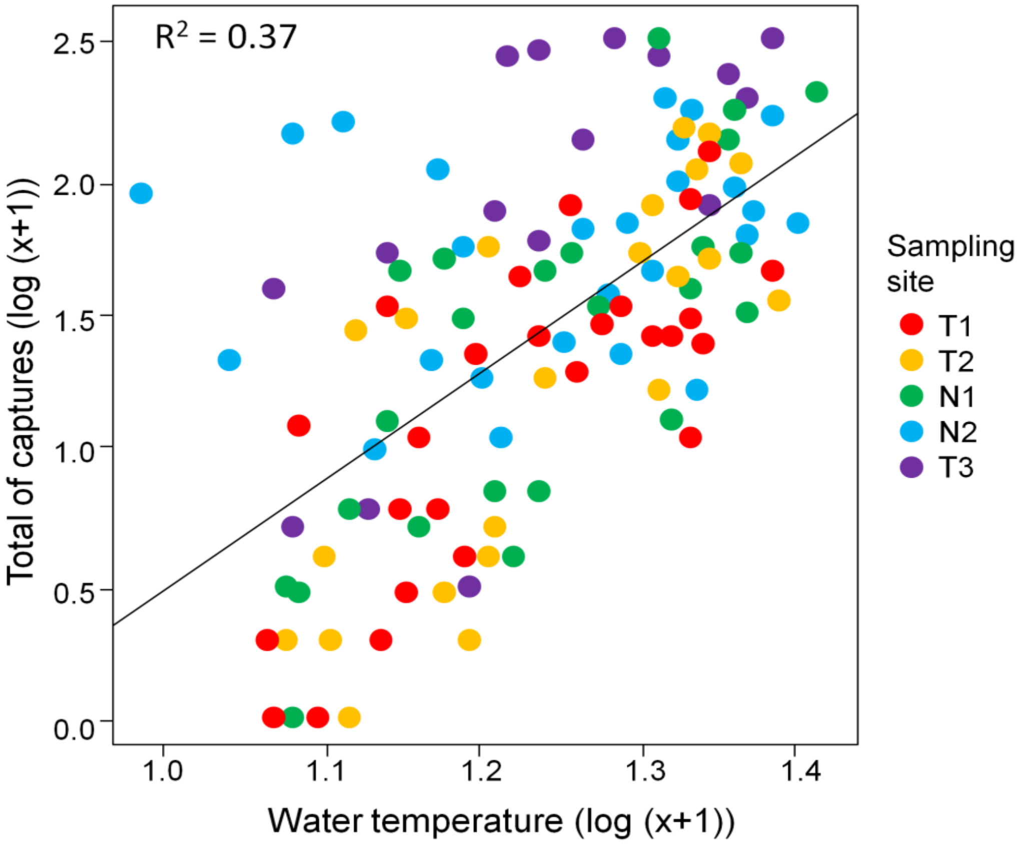

Figure 3.8 Relationship between water temperature and total captures inside the fishways (T1, T2, T3, N1 and N2) monitored in the main stem of the Segura River (south-east of the Iberian Peninsula). Black line represents a significant linear regression ($p < 0.05$).

3.4 Discussion

The present two-year study assessed the use of different multi-species fishways built in a heavily modified Mediterranean-type river, providing insightful data on the response of the whole fish assemblage. After evaluating different typologies of fishways, our results showed an early and extended use of both nature-like and technical typologies mainly by the dominant river-resident cyprinids, although they displayed differences in their use towards fishway types and/or temporal periods.

Exhaustive temporal monitoring for two complete annual cycles allowed us to verify that the most representative fish species present in the study area (the four target cyprinids) were regularly detected inside the assessed fishways, but size diversity and captures varied between typologies and periods. The fish captures inside fishways were associated with the fishway typology and probably with the downstream habitat characteristics. Water temperature plays the most important role among the environmental variables to explain variability in fishway use by fish species. We also detected use-related differences between the expected non-movement and movement periods, probably promoted by the use of fishways as migration corridors, such as by potamodromous fish. Moreover, although most of the species present in the study area are considered non-migratory (e.g., *G. lozanoi*, *A. alburnus*, *L. gibossus*), all of them make short-distance movements between different habitats for population persistence (Mclaughlin et al., 2013; Tummers et al., 2016). Furthermore, fishways seem to have provided new compensatory habitats for small and juvenile individuals throughout the annual cycle, mostly for reophilics such as gudgeons (*G. lozanoi*) and nases (*P. polylepis*) in nature-like fishways and for limnophilics such as bleaks (*A. alburnus*) in some technical types of fishways.

3.4.1 Fishway use by fish assemblage: technical vs. nature-like fishways

The monitoring programme of the present study started during the first three months when fishways were operational, so we were able to record the first fish use events for all the assessed fishways. Most of the species were present since the first fish survey, so the early use of these new infrastructures by different species could initially indicate the successful ecological functionality of these multi-species fishways (Tummers et al., 2016). In addition, the very high capture rates for the target cyprinids, especially two representative potamodromous fish of the Iberian rivers (i.e., *L. sclateri* or barbel and *P. polylepis* or nase) could be used as indicators of a well-implemented restoration tool (Benitez et al., 2015). This would be in concordance with the main results of the study of passage performance previously developed in two of the fishways, T2 and T3, with the two mentioned target fish (Sanz-Ronda et al. 2019).

Two of the technical fishways studied, T2 and T3, were the most successful in terms of species richness, with T3 also showing the highest captures, probably conditioned by isolated sampling events with significantly high abundance of two small resident cyprinids: gudgeon and bleak. We detected almost the totality of the potential fish species (eight out of nine species present in downstream reaches) inside these two technical fishways. Consequently, all large non-native limnophilics with different swimming abilities were captured inside them (i.e., *C. carpio*, *S. lucioperca, M. salmoides* and *E. lucius*), although the dominant captured fish were the small limnophilic bleak and the rheophilic gudgeon, which are also non-native to the study area. This occurrence of a wide variety of species supports that, despite technical fishways not offering the most suitable environmental and hydraulic characteristics for low-fitness species (tight entrance, higher slope, etc.), pool-type designs focused on multiple species (i.e., including large resting areas) could be as effective as nature-like fishways (Branco et al., 2013; Katopodis and Williams, 2012; Noonan et al., 2012).

However, in the present study, SNBO types (submerged notch with a bottom orifice) were significantly less used by fish species in terms of captures, which might suggest lower selection of this typology as a compensatory habitat, with fish using them mostly as migration corridors. Assuming a similar fish assemblage composition and structure in the whole river reach of the present study, the low rate of fish captures for this pool-type design could be explained by the hydraulic complexity of the adjacent reaches just downstream of the T1 and T2 fish passage facilities, with fishways in these sites probably used only for fish movements, while T3 is also used as a compensatory habitat. These results are in concordance with the idea that although barbels are known to prefer submerged notches (Silva et al., 2020), the implementation of vertical slot types is preferable because this typology choice provides more stable hydraulic conditions that could be relevant to the ascent of less fit species (Bravo-Córdoba et al., 2021, 2018b; Romão et al., 2017; Travade and Larinier, 2002). In addition, this technical design could offer the opportunity to control some non-native fish if selective traps are included (Pratt et al., 2009; Starrs et al., 2015; Stuart et al., 2006). In fact, our study shows the efficacy of vertical slot type for the massive capture of some non-native small cyprinids, which could indicate the potential use of these structures in fish population control.

Inside nature-like fishways, we detected five or six out of the potential fish species inhabiting downstream river reaches. Our results revealed that the bypass type was preferred by potamodromous fish but was also largely used by gudgeons (see Figure 3.2). However, large exotic species such as *C. carpio, M. salmoides* and *S. lucioperca* were not detected. In fact, nature-like designs attempt to mimic riffle/run habitats of natural river reaches, thus offering more fish-friendly environmental conditions (e.g., substrate and vegetation) and hydraulic characteristics (e.g., lower depth and higher mean velocity, with resting areas behind boulders and rocks) than technical types for rheophilic fish (Katopodis et al., 2001; Larinier, 2002; Pander et al., 2013). In addition, although nature-like fishways provide different environments and diverse hydraulic features that facilitate fish passage for a wider range of fish sizes and swimming abilities (Bunt et al., 2012; Katopodis and Williams, 2012), mimicked Mediterranean hydraulic features could be harder for limnophilic fish to pass (Colin et al. 2018). These results were reflected in the absence of captures of large limnophilics inside N1 and N2 in our study. According to this pattern, nature-like fishways have been proven to be used for rheophilic fish, though their use as compensatory habitats is precluded for medium- and large-sized non-natives. Moreover, other studies have even shown passage problems for large non-natives (e.g., *Exos lucius*) in this type of fishway (Calles and Grendberg, 2007). This result could be especially relevant in semiarid climatic regions where altered environments promote the establishment and spread of non-native fish species. In these highly invaded rivers, the implementation of multi-species fishways must take into account the risk of invasion when reconnecting human-isolated river reaches (Radinger and García-Berthou, 2020; Starrs et al., 2015).

3.4.2 Ecological contributions of multi-species fishways

In general, rheophilic fish were more frequently detected during the defined movement period inside fishways, even more significantly inside SNBO types where occurrence data could suggest their preferred use as migration corridors for these fish species. In the case of barbels, more than 70% of their captures inside the fishways were concentrated in the movement period (spring and summer), which could be an indicator of their effectiveness in facilitating migration movements for this endemic Iberian fish. Moreover, although movements of large barbels could also be detected during autumn (Ordeix, 2017; Romão et al., 2019; Sanz-Ronda et al., 2021), we mostly observed larger reproductive individuals during the defined movement period. Nase, a water column fish, showed significantly higher occurrences during the movement period only inside the SNBO type, which may be due to its swimming behaviour (Sanz-Ronda et al. 2016 and 2019). However, large individuals remained inside nature-like fishways during the movement period, which could also confirm successful migratory movements across these bypasses.

Each studied fishway differed from its adjacent downstream and upstream river reaches, providing lower water depth, smaller channel width and greater habitat variability inside them. Following these features, they probably provided key and compensatory micro- and mesohabitats that offer additional resources as well as refuge for small sizes and juvenile rheophilic fish to face flow variations throughout the year (Pander et al., 2013). According to their inner characteristics, nature-like fishways are also expected to be colonized more rapidly by aquatic invertebrates, vegetation and riparian plants, increasing the availability of resources such as food and refuge, but detailed information on naturalisation of the fishways is needed to confirm potential microhabitat effects (Boavida et al., 2010; Ramler and Keckeis, 2019).

The rheophilic gudgeon, non-native in the study area, is considered a non-migratory fish, but short-distance movements could be observed during its reproductive period (Amat-Trigo et al., 2013; Benitez et al., 2015). Our results showed high occurrences and larger individuals during the movement period inside all fishway types, which could suggest their successful use as migration corridors. However, most of the gudgeon captures were also concentrated during the non-movement period, especially inside nature-like fishways and vertical slots. An important use of nature-like fishways by other rheophilics, such as barbel and nase, outside the movement period was also observed, showing their highest captured individuals inside this typology. All of these results could confirm the majority use of this type as new compensatory habitats for rheophilics. The habitat conditions inside nature-like fishways seem to be similar to those in small upstream tributaries of the Segura River basin, where the species show high population density (Martínez-Morales et al., 2010).

The non-native bleak was the unique target fish that showed higher captures during the non-movement period inside the five fishways and also displayed significant differences in the size of captured individuals between periods. This limnophilic fish is considered not to make migratory movements, so the temporal pattern found in this study was probably conditioned by its characteristic movements forming high-density shoals not related to spawning movements, but by its high capacity to colonize new fluvial sectors (Amat-Trigo et al., 2019). In fact, some peaks of captures inside fishways as well as GLMs results, revealed that technical vertical slot type T3 was the most differentiated as a consequence of its correlation with the captures of this limnophilic fish, although gudgeon also added a relatively high number of captures during the non-movement period. Upstream movements by gudgeons were previously observed in other types of pool-weir fishways (Aparicio et al., 2012). Therefore, these findings confirmed that fish with no reproductive movements were using these multi-species infrastructures (Benitez et al., 2015).

The results from the mark-recapture surveys provided evidence on the up- and downstream fish-passability of the three rheophilic target fish. Although the results of fish passage data were scarce, they could be indicative of the successful fishway negotiation of these cyprinids, including endemic barbel, which would help to avoid the isolation of fish populations by recovering their longitudinal connectivity (Branco et al., 2013; Santos et al., 2005; Sanz-Ronda et al., 2021). Despite the results displaying high recapture rates inside all fishways, nature-like type N2 showed higher values, with fish even captured four times, which reinforces the idea that this typology provides more availability of key habitat that offers compensatory resources such as food or fish refuge (Katopodis and Williams, 2012; Pander et al., 2013).

River flow discharge and water temperature are considered the main drivers of fish behaviour (Cooke et al., 2012; Mims and Olden, 2012). In this study, capture variability was significantly related to water temperature and river flow discharge in the case of barbels. Water temperature is widely recognized as the main factor conditioning cyprinid movements in the Iberian Peninsula and could be driving fish movements for most of the studied species (García-Vega et al., 2021; Rodriguez-Ruiz and Granado-Lorencio, 1992; Santos et al., 2012; Starrs et al., 2015). As indicated by our GLMs analysis, we can confirm the strong influence of the water temperature on fishway use by fish. This factor generally covaries with flow regime (Murchie et al., 2008) and is generally strongly influenced by flow patterns in regulated rivers (Fornaroli et al., 2020; García-Vega et al., 2021), as reflected in our results by the significant correlations (Table 3.5).

The flow discharge inside the fishways was designed to maintain a relatively constant flow over time, causing minimal influence on fish passage (Romão et al., 2019). Flow discharge inside the fishways did not show a significant effect on total captures inside fishways (Table 3.6), and the high occurrences of small sizes and juveniles could indicate that there are no related effects on fish behaviour, thus highlighting successful hydraulic operation. In addition, we observed that flow inside the fishway is relatively independent of river flow discharge, which is essential to maintain suitable hydraulic conditions for fish ascent (DVWK, 2002) and is especially important in Mediterranean rivers strongly affected by flow fluctuations throughout the year (Bravo-Córdoba et al., 2021; Romão et al., 2017). Both the reduction of problems related to flow variability and the highest flow stability inside the fishways could also favour their use as refuges (micro- or mesohabitats), mainly for juveniles of cyprinids, which are especially important in heavily regulated rivers (Miró et al., 2018; Silva et al., 2020). In fact, the middle part of the Segura River basin, where this study was conducted, shows a reverse flow regime, with high flows in summer and lower flows in autumn and winter, as well as high daily flow variability due to weir operations (Sánchez-Pérez et al. 2020, Amat-trigo et al. 2021). In this fluvial sector, the riparian habitat showed a poor conservation status with reduced lateral connectivity and modified bank conditions mainly due to severe channel incision, which likely results in huge reductions in habitat availability and suitability (Elosegi et al., 2010). Thus, it could be argued that during high flow discharges, larvae and juveniles are more likely to be washed away due to the lower availability of refuges or sheltered habitats. Therefore, the usefulness of fishways as key habitats for juvenile cyprinids is increased in river sections with marked alterations of the flow regimes.

3.5 Conclusions and recommendations

Multi-species fishways could be an effective tool in connectivity restoration in strongly regulated Mediterranean rivers if they are well designed and implemented, which would not only be suitable for migration and other kinds of movements necessary for river fish populations but also play an important role as compensatory habitats for fish with different biological requirements and behaviours. For the successful implementation of multi-species fishways, it is essential not only to know their effectiveness for native species but also to understand how the whole assemblage will use these infrastructures, especially in areas highly invaded by exotic fish.

Technical fishways could be as efficient as nature-like fishways in highly regulated rivers, as they are logistically easier to implement (Branco et al., 2013; Katopodis and Williams, 2012; Larinier, 2008; Noonan et al., 2012) and, consequently, they are most frequently built (Silva et al., 2020). However, nature-like bypasses offer a better alternative for a more holistic approach of river restoration to try to re-establish a more natural dynamic in river systems (Pander et al., 2013). In the present study, nature-like fishways seem to be the most appropriate design to be implemented in Mediterranean rivers as long as the location conditions allow adequate implementation. Because this design is mostly used by the most abundant resident river fish (rheophilic cyprinids) and because they were not apparently used by non-native fish (especially large limnophilics), nature-like fishways should be considered more intensively in river restoration projects. Nevertheless, more studies in different river basins will be necessary to establish the behaviour of non-native limnophilic fish in this type of infrastructures.

Restoring longitudinal connectivity in intensively regulated rivers is crucial to achieve the good ecological status established by the European Water Framework Directive (European Commission, 2000), but the risk of spreading invasive species must be thoroughly considered in ecological restoration planning (Pratt et al., 2009; Radinger and García-Berthou, 2020; Terêncio et al., 2021). Exhaustive long-term monitoring is also essential in areas with high intra- and interannual variability, such as Mediterranean rivers, to increase the knowledge of fishway use under changing conditions (Benitez et al., 2015; García-Vega et al., 2021).

Special attention to flow design inside fishways is essential for successful operation under the strong flow variability in Mediterranean rivers, but also as a tool that facilitates or precludes their use by certain fish, where flow effects could be used as a management measure of the fish assemblage structure (Starrs et al., 2015). In fact, captures inside technical type fishways in the present study could indicate the potential use of these infrastructures in non-natives control. Increased knowledge about the benefits and costs of multi-species fishways will help to achieve more successful results when restoration measures for river connectivity are implemented, and it could inform water and wildlife managers to encourage appropriate policy-making processes.

Chapter 4

Passage performance of technical fishways by two Iberian potamodromous cyprinids in a Mediterranean-type river

Abstract

Endemic freshwater fish from semi-arid environments are among the most threatened species in the world due to water overexploitation and habitat fragmentation problems. Stepped or pool-type fishways are used worldwide to re-establish longitudinal connectivity and mitigate fish migration problems. Many of them are being installed or planned in rivers of semi-arid environments, however, very few studies about fish passage performance through pool-type fishways has been carried out to date on these regions. The present work focuses on the passage performance of two potamodromous cyprinids endemic of these regions, with different ecological and swimming behaviour: Southern Iberian barbel (*Luciobarbus sclateri*) and Iberian straight-mouth nase (*Pseudochondrostoma polylepis*). These are assessed in two of the most common types of stepped fishways: vertical slot and submerged notch with bottom orifice fishways. Experiments were carried out during the spawning season in the Segura River (south-eastern Spain), using a passive integrated transponder (PIT) tag and antenna system. Ascent success was greater than 80%, with a median transit time lower than 17 minutes per meter of height in all trials, and for both species and fishway types. Results show that both types of fishways, if correctly designed and constructed, provide interesting alternatives for the restoration of fish migration pathways in these regions.

4.1 Introduction

Rivers in semi-arid environments are subjected to strong seasonal variability, for instance, long drought periods alternated with large but brief floods (Gasith and Resh, 1999; Seager et al., 2019). Thus, water resources are strongly exploited, and rivers are highly affected by barriers and flow regulation (Grill et al., 2015; Smith et al., 2014). Transverse barriers, such as dams, weirs and gauging stations, and the involved habitat fragmentation, are considered the main threats to fish fauna worldwide (Lucas et al., 2001; Nilsson et al., 2005), especially in semi-arid regions (Coad, 2006; Maceda-Veiga, 2013; Hermoso and Clavero, 2011). Moreover, near-future scenarios suggest more water demand and the exacerbation of human stressors (Vörösmarty et al., 2000; Solomon et al, 2007). In these areas (i.e., from the circum-Mediterranean region to Central Asia), freshwater fish fauna present a high degree of endemism and are characterized by a low number of families, with most of the species belonging to the Cyprinidae family (Clavero et al., 2004; Kottelat and Freyhof, 2007; Jouladeh-Roudbar et al., 2015; Freose and Pauly, 2019). The most abundant species are barbels (genus Barbus and Luciobarbus) and nases (genus Chondrostoma, Pseudochondrostoma, and Parachondrostoma). Both rheophilic potamodromous cyprinids are under different levels of threat according to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Smith et al., 2014; Doadrio, 2002). This ichthyofaunistic group is an important link for the trophic interactions within the ecosystem and inhabits the entire length of the river, migrating during the spring in order to reproduce in shallow waters with gravel and moderate current velocity (Kottelat and Freyhof, 2007; Collares-Pereira et al., 1996). Two representative potamodromous fish species of the Iberian semi-arid region are the Southern Iberian barbel (*Luciobarbus sclateri* (Günther, 1868)) and the Iberian straight-mouth nase (*Pseudochondrostoma polylepis* (Steindachner, 1864)) (Doadrio et al., 2011). Both species are common in the southern and eastern Iberian Peninsula and they show different ecological traits and swimming behaviour (Torralva and Oliva-Paterna, 1997). The Southern Iberian barbel is defined as a sentinel species (Encina et al., 2006) in this region, and it is a large-bodied benthic fish that lives in slow water velocity habitats. Iberian straight-mouth nase is a medium-bodied water column fish that inhabits running waters (Doadrio, 2002).

River connectivity is an essential requirement for the effective functioning of freshwater ecosystems, and in particular for allowing fish to complete their life cycles (Pringle, 2003; Vannote et al., 1980). The longitudinal connectivity for fish is usually restored by different types of fishways. Technical pool-type or stepped fishways are the most used designs around the world (Clay, 1994; DVWK, 2002), including semi-arid regions (Lynch, 1956; Elvira et al., 1998; Verep et al., 2016; Pervin, 2017). Nevertheless, in northern Africa, the eastern Mediterranean area and Central Asia, fishways in cyprinid rivers are still very scarce (Comoglio, C., Mimeche, F. and Koutrakis, E.T. 2019. *Pers. comm.*). These types of fishways consist of pools connected by cross-walls with slots, notches, and/or orifices, which divide the total height of an obstacle into smaller drops to ensure that the hydraulic conditions inside, are in the range of the physical capacities of fish fauna, and thus, enable their passage (Larinier, 2002; Fuentes-Pérez et al., 2017). The most common designs in the Iberian Peninsula are vertical slot (VS) and submerged notch with bottom orifice (SNBO) fishways (Elvira et al., 1998; Silva et al., 2004).

On one hand, VS fishways allow fish movements at any desired depth through the slot and they tolerate variations in the upstream water levels better than SNBO, although they need more discharge than SNBO to get the same depth. In the other hand, SNBO can work with greater slopes and a wider range of design discharge, and they always ensure a minimum depth in the pool. In SNBO, the bottom orifice allows benthic fish passage, although it can be easily clogged by debris (DVWK, 2002; Larinier, 2002).

The suitability of both VS and SNBO has been previously probed for North American and Central European cyprinids (Noonan et al., 2012), and even for some northern Iberian cyprinids, such as Iberian barbel (*Luciobarbus bocagei* Steindachner, 1865) and Northern straight-mouth nase (*Pseudochondrostoma duriense* Coelho, 1985) (Sanz-Ronda et al., 2016, Bravo-Córdoba et al., 2018a, 2018b). Similar ascension and swimming behaviours are usually assumed for other species of the same genus or family, and thus fishway design criteria of one region are usually extrapolated to other river basins (DVWK, 2002; Branco et al., 2017; Sanz-Ronda et al., 2017a, 2017b). Nevertheless, species have evolved by adapting to different hydraulic regimens and climate conditions. Semi-arid southern Iberian rivers are usually more unsteady and warmer than northern ones. Fishways assessments in this region are still scarce. Hence, studying passage performance of southern fish species will provide important information about their ascent abilities, and in general will improve fishway design.

Passage performance depends on the interactions between physical (geometric and hydraulic features of the fishway), biological (fish behaviour, swimming ability, age, sex, physiological status), and environmental (water temperature, fishway discharge) parameters (James and Johnston, 1998; Plaut, 2002; Clough et al., 2004; Pedersen et al., 2008). Standardized passage metrics based on movement theory usually consider fish ascent success, transit time, and motivation (definitions in section 4.2.4) to quantify passage performance (Castro-Santos et al., 2009; Castro-Santos and Haro, 2010; Cooke and Hinch, 2013), and also to compare different types of fishways or to understand the swimming behaviour of different fish species (Sanz-Ronda et al., 2016; Bravo-Córdoba et al., 2018b; Romão et al., 2018).

The present study focused on the passage performance of two potamodromous cyprinids from a semi-arid region of the Iberian Peninsula, Southern Iberian barbel and Iberian straight-mouth nase, in the two main typologies of pool-type fishways: VS and SNBO. Specifically, ascent success, passage time, and motivation of target fish were analyzed to determine: (1) if both types of fishways are suitable for them; (2) if there were differences in passage efficiency between fishway types and fish species; and (3) to understand the influence of hydraulic and biometric parameters on ascent metrics. This information will help biologists and engineers in fishway design, implementation, and management decisions in many semi-arid watercourses inhabited by species with similar behaviour.

4.2 Material and methods

4.2.1 Study area and experimental sites

The Segura River basin, placed in the south-east of Spain, is one of the most arid European regions, with an average annual temperature of 18 ºC, scarce rainfall (approximately 300 mm/year), and intense surface and groundwater overexploitation for irrigation (Grindlay et al., 2011). The experiments were carried out in two fishways located in the middle part of the Segura River: VS in the El Jarral weir (Universal Transverse Mercator –UTM-Grid Zone 30 North; European Terrestrial Reference System 1989 -ETRS 89-, X: 640577, Y: 4229308; Abarán, Murcia) and SNBO in the

Post-trasvase weir (UTM30 ETRS 89, X: 613788, Y: 4235661; Calasparra, Murcia) (Figure 4.1). Both fishways are placed in small weirs for combined irrigation and hydropower production. The distance between them is 54 km approx. and they share similar environmental characteristics (flow discharge, substrate, vegetation, and fish population). The fish assemblage in the main stem of the Segura River is severely altered due to the modification of the hydrological regime for irrigation and the introduction of non-native invasive species (Oliva-Paterna et al., 2014). In this part of the basin, among the most abundant fish are the potamodromous migratory species: the native Southern Iberian barbel and the translocated Iberian straight-mouth nase (hereafter referred to as barbel and nase, respectively) (Oliva-Paterna et al., 2014).

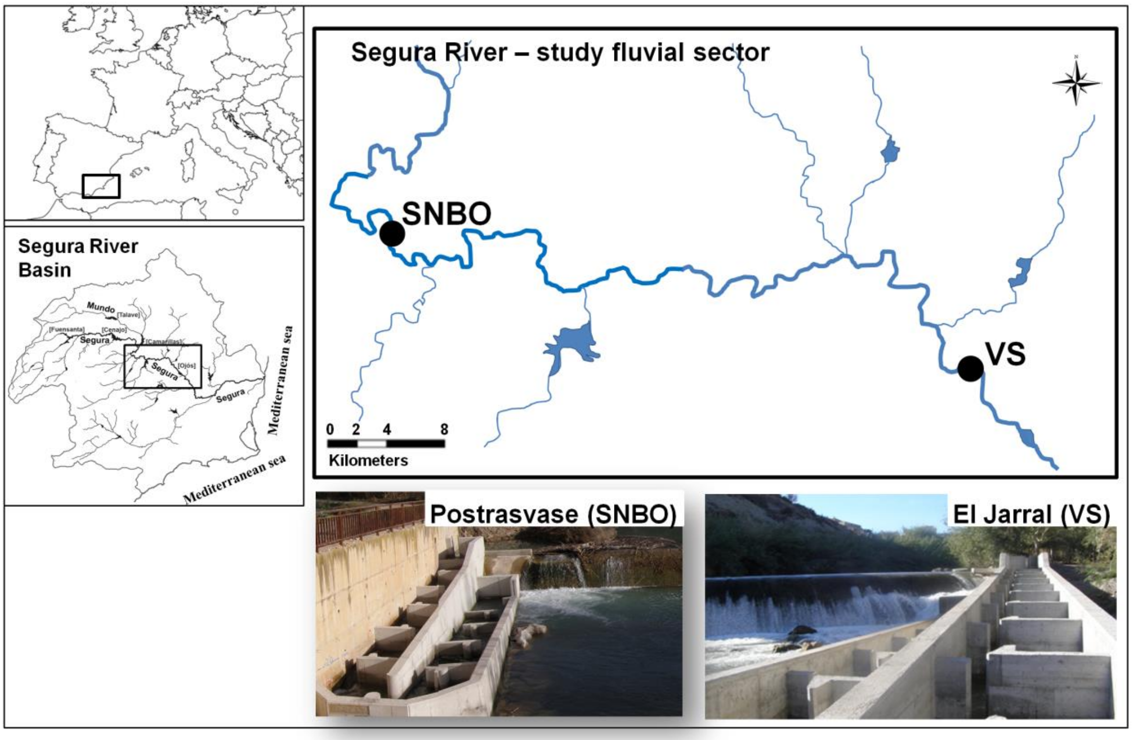

Figure 4.1 Location of sampling sites (●) in the Segura River basin and its location in the south-east of the Iberian Peninsula. (SNBO) submerged notch with bottom orifice type and (VS) vertical slot type.

Both fishways were designed and constructed as a part of the Segura-Riverlink LIFE12ENV/ES/001140 project (Oliva-Paterna et al., 2016), following the standard design guidelines (DVWK, 2002; Larinier, 2002) and considering the geometrical and hydraulic recommendations for cyprinids (Figure 4.2 and Table 4.1). Fishway bottoms were covered by substrates from the riverbed to increase roughness, and discharge can be regulated by a sluice gate located in the flow entrance. At each fishway, a section with a 1.80 m head (difference between the headwater and tailwater levels) was selected for the trials.

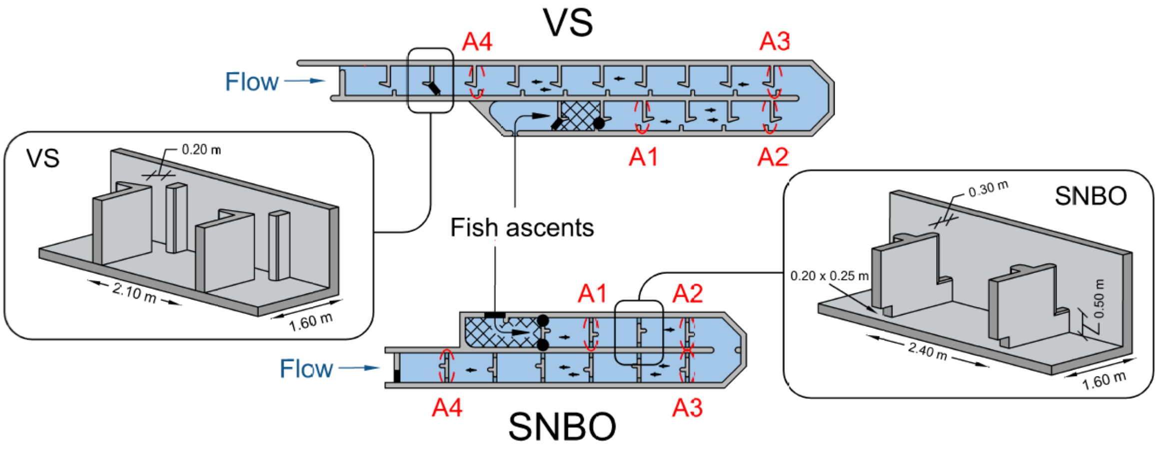

Figure 4.2 Experimental set-up: (SNBO) submerged notch with bottom orifice type and (VS) vertical slot type.. Black cross-walls: closing mesh (start and finish of the test section); black circles: closing mesh during adaptation period; mesh over the pools: adaptation pools; dotted circles: antennas with their position number (antenna 1 -A1-, antenna 2 -A2-, etc., being 1.80 m, the relative height between A1 and A4 for both fishways); arrows indicate flow and fish ascent directions.

Table 4.1 Mean geometric and hydraulic variables for the studied vertical slot (VS) and submerged notch with bottom orifice (SNBO) fishways. Range of values in brackets.

Variables	VS	SNBO
Pool dimension (length x width)	2.10 m x 1.60 m	2.40 m x 1.60 m
Slope	6.52%	7.31%
Number of pools between A1 and A4	11	8
Width of the slot/notch[1]	0.23 m (0.20 - 0.23)	0.31 m (0.31 - 0.36)
Height of the notch sill[1]	NA	0.49 m (0.45 - 0.52)
Bottom orifice size (length x width)	NA	0.20 m x 0.25 m
Drop between pools[1]	0.15 m (0.14 - 0.19)	0.19 m (0.13 - 0.24)
Mean water depth[1]	0.91 m	0.99 m
Flow discharge[2]	0.29 m^3/s	0.31 m^3/s
Volumetric Energy Dissipation	118 W/m^3 (110 - 149)	148 W/m^3 (102 - 181)
Water velocity at the slot/notch[3]	1.38 m/s (1.10 - 1.47)	1.24 m/s (0.80 - 1.36)
Water velocity at the orifice[3]	NA	1.72 m/s (1.42 - 1.93)

[1] Measured with a total station (model Leica TC307, Heerbrugg, Switzerland) or measuring tape ±0.01 m.

[2] Calculated according to Fuentes-Pérez et al., (2017).

[3] Direct measurements with a propeller flow meter ±0.01 m/s (model 2100, Swoffer Instruments Inc., Wummer, WA, USA).

Note: NA = not applicable.

The boundary conditions imposed by the river at the entrance of the fishway (water lever downstream) during the trials influenced the lower part of the fishway, producing non-uniform conditions with backwater profiles (more evident in the SNBO), increasing the water depth in the most downstream pools and reducing the water drops, and thus obtaining lower values of water velocity and energy dissipation (Fuentes-Pérez et al., 2017; Noonan et al., 2012).

4.2.2 Fish capture, tagging and handling

A passive integrated transponder (PIT) tag and antenna system was used to study fish movements. Trials were performed from 3 to 8 May 2017, within barbel and nase reproductive migration periods. Fish were captures by electro-fishing (1800W generator, working voltage between 200 and 350V, 2–3 A) one day before trials in river reaches upstream of each fishway. There were unpassable barriers between the capture areas and the experimental fishways. Once captured, fish were anesthetized with tricaine methanesulfonate (MS-222, 0.1 g/L), measured (fork length, ±0.1 cm), weighed

(±1 g), and tagged with a PIT-tag (Table 4.2). A PIT-tag is an encapsulated microchip used for radiofrequency identification (RFID). PIT-tags were introduced into the intra-peritoneal cavity of the fish through an incision posterior to the left pectoral fin (Castro-Santos and Vono, 2013). As the weight of each tag must be lower than 2% of the fish weight, two sizes of PIT-tags were used: 23 mm long and 3.65 mm diameter, and 12 mm long and 2.12 mm diameter (TIRIS model RI181 TRP-WRHP; Texas Instruments). This method shows negligible effects on growth, survival, and behaviour of many species (Ostrand et al., 2012) and is very common in fish movement studies (Cooke et al., 2013).

Afterwards, fish were transported in aerated water tanks (100 L) and subsequently stabled in two similar groups per fishway (mix of barbel and nase) for acclimation inside cages: groups VS1 and SNBO1 in a pool in the fishway, and groups VS2 and SNBO2 in the river near the fishway. Prior to the start of the trials, each group was confined to the initial pool (the most downstream; Figure 4.2) by two closing mesh areas and a low fishway flow (50 L/s), to ensure adaptation and avoid stress or fatigue. Fish were not fed during experiments, although they could access natural food sources drifting into the cages or on the bottom. No fish died during or after the tagging process and trials.

Fish were treated in accordance with the European Union Directive 2010/63/UE on the protection of animals used for scientific purposes and Spanish Act RD 53/2013, following the ethical guidelines of Murcia University and with the approval of the Government of Murcia (Regional Government on Natural Resources and Water Management Authority), under authorization AUF20150077. Moreover, significant efforts were made to minimize stress and fish were released after the experiments.

4.2.3 Trials

A system of antennas was used in both fishways to detect the movements of the PIT-tagged fish. Four antennas were installed inside each fishway at a total head of 1.80 m (Figure 4.2), covering the slot in VS and the notch and the orifice in SNBO, with a detection range of ±20 cm distance from the antenna. Each antenna was connected to a reader (Half Duplex multiplexer reader, ORFID®, Portland, Oregon, USA) programmed to send and receive information at 14 Hz (3.5 Hz or 0.29 s per antenna).

At the start of a trial, the fishway gate was open, allowing usual operating flow (Table 4.1), and the closing mesh in the starting pool was removed. Therefore, fish were allowed to ascend volitionally but they could not escape from the fishway due to the presence of the closing meshes in both the lower and upper zones of the experimental area (Figure 4.2). If a fish reached the uppermost pool, it had two options: remaining there or descending. Two 16-hour trials (from 8:00 to 24:00 h) were attempted in each fishway, one for each fish group; thus, a fish participated in only one trial (Table 4.2). Prior to trails, other fish that remained in the fishways were removed before releasing experimental individuals from each group.

Water level and temperature were monitored at 30 min. intervals (Orpheus Mini, OTT Hydromet GmbH, Kempten, Germany), with small variations observed during the trials (16–19 ºC in the VS group and 14–17 ºC in the SNBO group). The weather was sunny and cloudless during the experiments.

Table 4.2 Fish samples used to conduct trials inside vertical slot type (VS) and submerged notch with bottom orifice type (SNBO).

Fish group	Species	n	Length (cm)		Weight (g)		K	
			Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
VS1	Barbel	36	20.0 ± 4.2	11.2- 28.2	133 ± 72	21- 298	1.51 ± 0.17	1.24 - 2.18
	Nase	23	16.5 ± 1.7	13.5 - 20.0	44 ± 18	26 - 92	1.11 ± 0.11	0.95 - 1.34
VS2	Barbel	36	19.0 ± 3.3	15.6- 27.9	127 ± 66	56- 322	1.49 ± 0.10	1.23 - 1.73
	Nase	23	15.0 ± 1.6	12.8 - 19.6	40 ± 17	25 - 99	1.14 ± 0.12	0.96 - 1.42
SNOB1	Barbel[1]	36	17.9 ± 8.9	11.5- 43.7	166 ± 344	24 - 1326	1.55 ± 0.14	1.26 - 1.83
	Nase[1]	23	14.8 ± 2.8	11.6 - 19.6	39 ± 24	19 - 85	1.12 ± 0.08	0.98 - 1.22
SNBO2	Barbel[1]	36	15.3 ± 2.2	15.3- 20.0	56 ± 27	28- 130	1.49 ± 0.11	1.32 - 1.77
	Nase[1]	23	14.0 ± 3.3	14.0 - 20.5	35 ± 29	16 - 99	1.13 ± 0.18	0.92 - 1.45

[1] Barbels and nases from SNBO group were significantly smaller ($p < 0.05$) than those from VS group.
N = number of fish tagged with PITs.
SD = standard deviation.
K = condition factor (100 x weight/fork length).
barbel = *Luciobarbus sclateri*; nase = *Pseudochondrostoma polylepis*.

4.2.4 Data analysis

During each trial, fish were able to make several ascents. To separate the ascent movements from the exploratory ones, we considered a passage attempt as when a fish reached the second antenna (Figure 4.2, A2). The last detection at antenna 1 (Figure 4.2, A1) was considered as the starting time of the attempt. The attempts in which the fish reached the most upstream antenna (Figure 4.2, A4), were deemed successful; otherwise, they were deemed failures.

Ascent Analysis

Ascent performance was analyzed using two usual metrics (Sanz-Ronda et al., 2016, Bravo-Córdoba et al., 2018a, 2018b): (1) ascent success: percentage of fish that reached A4, in relation to the total number of fish that attempted it. It was analyzed by the chi-square test of independence; and (2) transit time: time taken to move from A1 (last detection) to A4 (first detection) in the fastest successful attempt. Cox proportional hazards regression (PROC, PHREG for SAS) with the Schoenfeld and Martingale residuals test for proportionality (Enders et al., 2017; Goerig and Castro-Santos, 2017) was used to identify differences in transit time by fishway type and species. In addition, survival analysis based on regression models (PROC, LIFEREG, and "Predict" Macro for SAS; (Allison, 2010)) was used to predict the transit time as a function of the significant covariates and the likelihood of ascent in a given time. The best fitting model was selected using Akaike's information criterion (AIC) (Akaike, 1974). Additionally, transit time was also expressed in minutes per meter of ascent height (min/m; transit time divided by total water level height ascended), allowing equivalent comparisons to be made between fishways or species. The median was used as the reference value due to the non-normal distribution of the data.

Motivation Analysis

Motivation was studied using three specific metrics: (1) attempt percentage: percentage of fish that attempted to ascend in relation to the total number of fish, analyzed using the chi-square test of independence; (2) number of attempts per fish: number of attempts staged by those fish, analyzed via Mann-Whitney test for median comparisons; and (3) attempt rate: proportion of attempts per unit of time, analyzed by Cox proportional hazards regression with Schoenfeld and Martingale test, which stratified the attempts and assessed the influence of fishway type and species. A differentiation between the first attempt (pre-attempt: first attempt in a trial) and the rest of the attempts was considered (Bravo-Córdoba et al., 2018b; Goerig and Castro-Santos, 2017).

Water temperature, depth, and fishway discharge were considered invariant during the time of the trials. All statistical analyses were performed using SAS® (Cary, NC, USA) (version 9.4) and Statgraphics Centurion (Statgraphics Technologies, Inc., The Plains, Virginia, USA) (version XVI.II) software.

4.3 Results

Fish groups in each fishway (VS1 *vs.* VS2, and SNBO1 *vs.* SNBO2) showed no significant differences in all ascent and motivation metrics ($p > 0.05$ in all cases). Therefore, data for both groups of fish in the same fishway were merged and processed together as a single group. In addition, the possibility of fatigue or learning during the ascent was analyzed by comparing the different attempts, but no pattern was observed to support those hypotheses.

4.3.1 Ascent analysis

The ascent success in all cases exceeded 80%, with significant differences between the type of fishways for barbel ($\chi^2 = 4.735$, $p = 0.032$) but not for nase ($\chi^2 = 1.609$, $p = 0.289$) (Table 4.3). The ascent success had no relation with the fish length for both species ($p > 0.05$ in all cases). Regarding transit time, significant differences were found between types of fishway and species. Barbel needed more time than nase in both fishways ($p = 0.052$ in VS and $p = 0.020$ in SNBO), and spent less time in VS than in SNBO ($p < 0.001$) (Table 4.3). However, nase spent similar median time in VS and SNBO ($p = 0.687$; Table 4.3).

Table 4.3 Results of the used metrics: percentage of attempts, number of attempts, ascent success, and transit time (1.80 m head). (SNBO) submerged notch with bottom orifice type; (VS) vertical slot type. Barbel = *Luciobarbus sclateri*; nase = *Pseudochondrostoma polylepis*.

Metrics	VS (N = 110)		SNBO (N = 56)	
	Barbel	Nase	Barbel	Nase
Attempt percentage	(58/66) 87.9%	(39/44) 88.6%	(30/39) 76.9%	(8/17) 47.1%
Median number of attemps	3 (1 - 16)	5 (1 - 12)	2 (1 - 6)	3 (1 - 6)
Ascent success	(55/58) 94.9%	(38/39) 94.8%	(24/30) 80.0%	(7/8) 87.5%
Median transit time[1]	(54) 12.5 min	(37) 8.0 min	(19) 26.3 min	(6) 9.3 min
Median transit time per meter of eight	6.9 min/m (1.1 - 274.5)	4.4 min/m (1.2 - 342.7)	16.6 min/m (4.6 - 405.3)	5.2 min/m (2.1 - 34.4)

[1] Fish number for the calculus of transit time differs from the total number of successful ascents due to some missing time data in antenna 1.

Note: The number of fish is indicated in brackets, except for the median number of attempts and median transit time per meter of height, where the range is in brackets.

Overall, fish length showed a significant relationship with transit time (p = 0.024). The best fitting predictive model between fish length and transit time was log-logistic survival regression. For barbel, fish length showed an inverse relation with the transit time (the longer fish, the less transit time) in both fishway types ($p < 0.05$) (Figure 4.3 and Table 4.4). For nase, while there were no significant differences in VS by fish length (p = 0.981), for SNBO the analysis results were significant (p = 0.016) but not conclusive, due to the low number of successful ascents (Table 4.3).

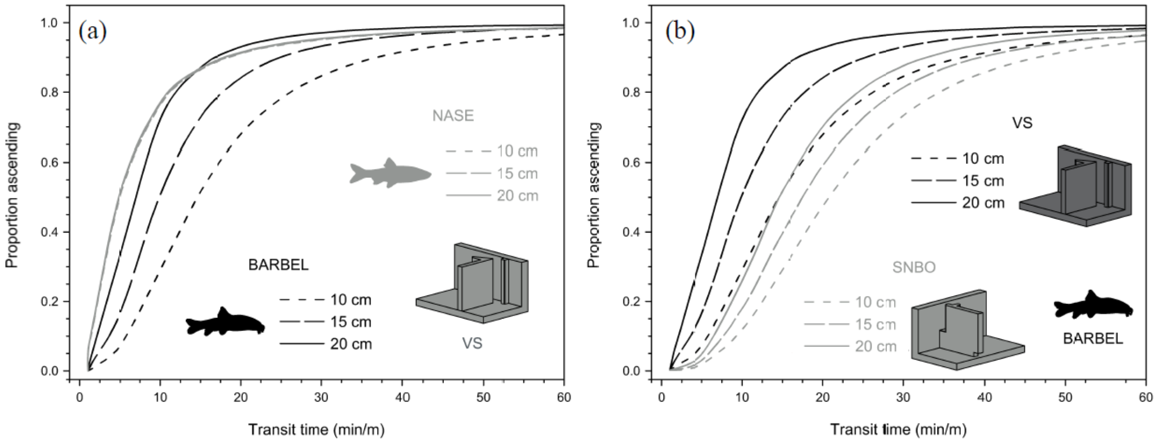

Figure 4.3 Predictive log-logistic survival model for the proportion of fish ascending (1 m of height exceeded) at a given transit time as a function of fish species and fork length (10, 15, and 20 cm) (a), and fishway type and length for barbel (which showed significant differences) (b). Note: VS = vertical Slot fishway; SNBO = submerged notch with bottom orifice fishway (SNBO); barbel = *Luciobarbus sclateri*; nase = *Pseudochondrostoma polylepis*. Nase in SNBO was not included due to the small sample size, which would not achieve a reliable predictive model.

Based on this model (Figure 4.3 and Table 4.4), the effect could be expressed approximately as reduction in transit time of 7.5% for barbel in VS ($\exp(\beta) - 1 = \exp(-0.078) - 1 = -0.075$) and 3.4% in SNBO per cm increase in fork length (considering the mean value of all fish). As an example of prediction, it could be said that 50% of barbel with a fork length of 15 cm would ascend 1 m height in 10 min. for VS and in 17 min. for SNBO. However, if the fork length was 10 cm, the transit time would be 14 min/m and 21 min/m, respectively.

Condition factor did not show significant relationships in any case for ascent analysis parameters.

Table 4.4 Estimation of the parameters of log-logistic survival model (μ= regression intercept; β = fork length (cm); σ = curve shape of the model) for the proportion of fish ascending at a given transit time as a function of fish length by fishway type (predictive model in Figure 4.3). (SNBO) submerged notch with bottom orifice type; (VS) vertical slot type. Barbel = *Luciobarbus sclateri*; nase = *Pseudochondrostoma polylepis*.

		VS			SNBO		
	Parameters	Coefficient	SE	*p*	Coefficient	SE	*p*
Barbel	Intercept (μ)	3.4601	0.5425	<0.001	3.3816	0.3209	<0.001
	Length (β)	-0.0784	0.0268	0.0034	-0.0350	0.0155	0.0239
	Shape (σ)	0.4236	0.0487		0.3657	0.0725	
Nase	Intercept (μ)	1.3243	1.7664	0.3578	5.4887	1.6238	<0.001
	Length (β)	-0.0028	0.1161	0.9809	-0.2365	0.0979	0.0157
	Shape (σ)	0.6001	0.0882		0.3033	0.1063	

SE = coefficient standard error.

4.3.2 Motivation analysis

The attempt percentage exceeded 75% in all cases, except for the nase in SNBO (Table 4.3). Nase performed a significantly lower number of attempts in SNBO ($\chi^2 = 11.987$; $p = 0.001$), while barbel showed no differences between fishways ($\chi^2 = 2.168$; $p = 0.074$) (Table 4.3). The median number of attempts per fish was higher for barbel in VS than in SNBO (3 *vs.* 2 attempts respectively; $p = 0.0165$), whereas there were no significant differences for nase (5 *vs.* 3 attempts; $p = 0.168$).

Regarding the attempt rate, there were significant differences in relation to the type of fishway ($p < 0.001$) but not between species ($p > 0.05$) for both pre-attempt rate and rate of the rest of attempts (Table 4.5 and Figure 4.4). The model fits indicated a significantly lower rate in SNBO than in VS (exp(β) − 1)·100 = 61.4% and 45.1% for pre-attempt rate and rate of the rest of attempts, respectively). This means that the likelihood of staging an attempt (hazards ratio = exp(β)) in SNBO is 38.6% lower for pre-attempt and 54.9% lower for other attempts.

Table 4.5 Estimation of the parameters of the Cox proportional hazards models (β: regression coefficient; HR: hazards ratio = exp(β); SE: standard error) for the attempt rate (pre-attempt and rest of attempts) in relation to the type of fishway and species for the reference factors submerged notch with bottom orifice fishway (SNBO) and nase (Iberian straight-mouth nase).

	Pre-attempt rate			Rest of attempts rate		
Parameters	β ± SE	*p*	HR	β ± SE	*p*	HR
Fishway	-0.951 ± 0.189	<0.001	0.386	-0.599 ± 0.149	<0.001	0.549
Species		0.595			0.124	

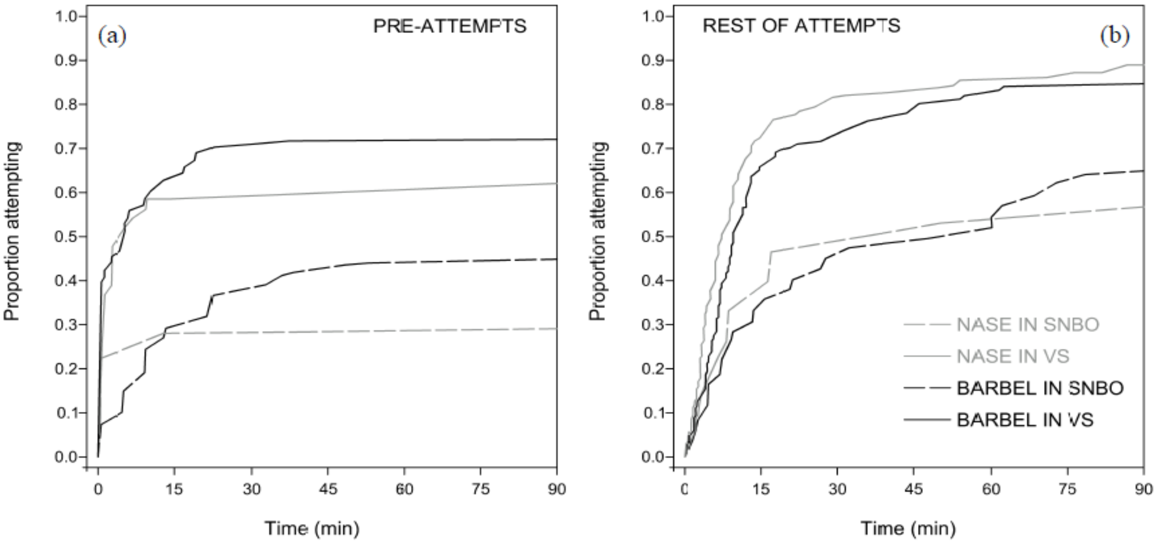

Figure 4.4 Kaplan–Meier curves for the proportion of fish attempting to ascend over time. Proportion for the pre-attempt rate (a), and proportion for the rest of the attempts (b).

4.4 Discussion

Most river basins in the Iberian Peninsula have a Mediterranean character, and therefore their native fish assemblages have evolved and are structured according to these semiarid environments (Encina et al., 2006; Alexandre et al., 2013a). These fish species have developed unique life-history strategies, which together with their high degree of rarity and endemicity, determine the high conservation interest presented by these faunal communities (Maceda-Veiga, 2019), as in other regions in the circum-Mediterranean area (Clavero et al., 2004; Jouladeh-Roudbar et al., 2015). Longitudinal connectivity disruption is one of the main alterations threatening fish populations (Smith et al., 2014; Coad, 2006; Doadrio et al., 2011), which is probably magnified in environmental scenarios, such as in the Iberian rivers in the Mediterranean area (Hermoso and Clavero, 2011).

The present work shows that mitigation measures, such as fishways designed according to criteria of their native fish species, could reduce river fragmentation impacts on these areas. Thus, the passage efficiency is presented for two potamodromous cyprinids, which are sentinel species from semi-arid environments with different swimming behaviour (Southern Iberian barbel and Iberian straight-mouth nase), through two of the most common pool-type fishways (vertical slot and submerged notch with bottom orifice fishways).

Results reveal that both species easily overcame both types of fishways. Overall, ascent success exceeded 80% in all trials, with a median transit time lower than 20 min/m for barbel and 10 min/m for nase, which would not imply an important migratory delay in any case. These values are similar to those for congeners *Luciobarbus bocagei* and *Pseudochondrostoma duriense* in the Duero River Basin (northern Iberian Peninsula (Sanz-Ronda et al., 2016, Bravo-Córdoba et al., 2018a, 2018b). Other studies for the close relative *Barbus barbus* in the Swiss Rhone River showed analogous transit time to ours in pool-type fishways (Grimardias, 2015). In general, fish size influenced the time needed to overcome the fishway, with longer times for the smaller fish, which is consistent with other fishway evaluations (Sanz-Ronda et al., 2016, Bravo-Córdoba et al., 2018a, 2018b; Assumpção et al., 2012). Longer fish had more body mass, as well large fins, which are very important as sources of propulsive forces to cross velocity barriers faster (Assumpção et al., 2012; Videler and Wardle, 1991).

Nevertheless, the study reveals significant differences in the variables that define the ascent and the motivation between fishways and species. Nase ascended both types with similar speeds and faster than barbel, meanwhile the latter ascended faster in VS and with higher percentage of success. Both species presented a higher motivation in VS.

Despite the fact that both barbel and nase are rheophilic potamodromous cyprinids (Doadrio, 2002) and usually coexist in the same river reaches, their swimming behaviour is quite different. Barbel is a benthic fish, with a robust body and greater swimming ability than nase for the same fork length (Sanz-Ronda et al., 2015; Ruiz-Legazpi et al., 2018). On the other hand, nase is a water column fish, with a slender body and good swimming and leaping aptitudes, which allow them to easily overcome obstacles (Sanz-Ronda et al., 2016).

Differences between our target species could be related to the date of the trials in relation to the reproductive periods of both (Torralva and Oliva-Paterna, 1997). Nase fish usually start spawning migration earlier than barbel, with the migratory peak in May, whereas barbel fish start at the beginning of June for the Segura River basin (Oliva-Paterna et al., 2019). Therefore, nase fish were expected to be more active than barbel during trials. However, ascent motivation was similar in both species, except for nase fish in SNBO, which showed lower attempt percentage. The upper water origin for the nase sample in SNBO compared to VS could partly explain the lower motivation for nase group. Colder water temperatures in upper parts of the basin could delay the maturation, and therefore the motivation, compared to the middle part in the main stream, and also the change of water quality from the capture area to the experimental site could influence fish behaviour (Bravo-Córdoba et al., 2018a). Moreover, although the difference between SNBO and VS in the experimental fish size was very small, individuals from the first type were significantly smaller, which could also have had an effect on fish motivation. We can assume that all of our experimental individuals were mature fish (Torralva and Oliva-Paterna, 1997), but in the case of nase fish, the first size of maturity was very close to the average size of the experimental specimens, which was more obvious in SNBO individuals (SIBIC, 2021).

Alexandre et al. (2014b) observed differences in swimming ability for *Luciobarbus bocagei* population depending on the river stretch of origin, which could be related to their genetic origin, but also to the habitat drivers from each population. Results from our study also showed slight differences in metrics, which could be explained by the origin of the populations and of each target species. Although nase has been completely adapted to the Segura River basin since its colonization (Torralva and Oliva-Paterna, 1997), it originally inhabits stretches of high–moderate velocity in their native source from the Tagus River, which are very different from the slow waters of the Segura River in the studied fluvial sector. Compared to barbel, it is best adapted to running waters, which could explain the faster transit times observed in the present study.

Although the hydraulic design parameters of both fishways were within the usual recommendations for cyprinids (DVWK, 2002; Larinier, 2002), some slight differences were found between them regarding the volumetric energy dissipation and water velocity. Changes in headwater or tailwater levels modify the hydraulic conditions in the entrance from the ones defined during the design process, causing non-uniform (backwater or drawdown) profiles (Fuentes-Pérez et al., 2017). During trials, the SNBO presented a more evident backwater profile, where the downstream pools, including the starting pools and the pools between A1 and A2, were more submerged (water drops < 0.15 m) than the other pools. This produced lower water velocity in the notches and orifices, and lower volumetric energy dissipation in pools (less than 1 m/s and 100W/m^3), which could have reduced the motivation due to the relationship with velocity in the notches (Goerig and Castro-Santos, 2017; Santos et al., 2012; Fuentes-Pérez et al., 2016), which would have increased the transit time. Larinier et al. (2002) recommends a speed higher than 1 m/s in the notches and slots for most species, and in the order of 2 m/s to 2.4 m/s for large rheophilic fish.

Therefore, fishway design projects for upstream migrating fish, specifically for potamodromous cyprinids in semiarid environments such as the Segura River, may provide an opportunity to develop safe, timely, and effective fish passage structures. Results obtained in this field study reveal that both fishway types, if correctly designed and constructed, offer interesting alternatives to mitigate the longitudinal connectivity problems for two sentinel cyprinids from those rivers in semi-arid environments. However, further research is necessary to improve the knowledge of the relationships between the behaviour and swimming ability of different fish species in the different fishway types and hydraulic scenarios. In fact, a complete and exhaustive fish-based monitoring assessment of the effectiveness of fishways should be an essential part of any project, in order to restore the longitudinal connectivity.

General conclusions

The purpose of this thesis was to study the response of the fish assemblage, and also of a sentinel species, to the effect of environmental stress factors, as well as to assess the use and ecological contributions of the implementation of fishways as a restoration tool for improvement of the river connectivity. As a result of this study, we can draw the following conclusions:

- The environmental stress factors present in the Segura River basin, as well as their magnitude and interaction, determine the composition and structure of the fish assemblage, selecting species with optimal biological traits to inhabit altered areas. The use of functional diversity indices has been shown to be more appropriate than traditional indices, solely based on species richness and abundance, to assess the effects of human impacts on the fish assemblage.

- The flow regime alteration is confirmed as a key factor structuring the fish assemblage in river reaches. Increased base flows and stability, as well as greater longitudinal fragmentation, induce an increase in non-native species and facilitate the proliferation of generalist species. As a result, the fish assemblage has shown a homogenization process due to loss of originality and specialization, despite an increase in its richness and species abundance.

- *Luciobarbus sclateri* showed age and growth variability only related to the flow regime alteration and the abundance of non-native fish species, suggesting that the magnitude of these human impacts could drive the expected biological responses along the longitudinal gradient. In contrast, the variability of the somatic condition and the size parameters was related to the longitudinal gradient, with greater values downstream where environmental stressors such as flow regime alteration, fragmentation and the abundance of non-native fish species were greater. In addition, the populations in river reaches downstream of large reservoirs showed significant impacts on their population structure with a lack of small individuals.

- *Luciobarbus sclateri* displayed high population trait plasticity along the longitudinal gradient, suggesting that this factor plays a key role in the adaptability of this species to inhabiting highly altered rivers. Nevertheless, significant effects of stress factors mainly related to flow alteration have been confirmed, providing information on the population response of this Iberian endemic species considered a sentinel species of Mediterranean rivers.

- This study revealed that multi-species fishways, designed for species with different ecological requirements, constitute a relevant ecological contribution for the dominant fish species (cyprinids) in river reaches of the Segura River basin. Increasing knowledge regarding the functionality of these infrastructures as measures to restore the river connectivity in Mediterranean basins is essential for the implementation of successful management strategies.

- Potamodromous cyprinids present in the fish assemblage used both nature-like and technical fishways, especially during their movement periods, indicating their success as migration corridors. Both multi-species designs were used as compensatory habitats for rheophilic fish, but also technical types for limnophilic species. As a result of the flow discharge and habitat characteristics inside the fishways, these infrastructures could be used as a refuge in river reaches with strong flow variability, being especially well-suited for juveniles and small individuals.

- Priority should be given to the implementation of nature-like fishways in Mediterranean rivers as a tool to restore the dynamics of fish populations, since this design better reproduces the natural conditions of river reaches, being more used by rheophilic cyprinids and less by large non-native limnophilic species. Even so, technical types, both vertical-slot and submerged notch with bottom orifice, if well designed and implemented, could provide a successful restoration measure, and could also be used as control traps for some non-native species, with the aim of reducing the spread of invasive fish.

- In short, the magnitude and the complexity of the interactions between environmental stressors in highly regulated Mediterranean rivers make it difficult to interpret the responses of fish populations and assemblages to human impacts. Increasing knowledge of the biological responses at river reach and regional scales as presented in this work should allow an improvement in the implementation of successful conservation and restoration measures.

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Appendix

Table S1. Sampling site location (UTM30 ETRS 89) with coordinates provided as Universal Transverse Mercator.

River	Site name	Chapter 1	Chapter 2	Chapter 3	Chapter 4	Altitude	UTM	
Mundo	Híjar	8	MU01	-	-	560	X: 586753	Y: 4265929
Mundo	Liétor	9	MU02	-	-	540	X: 591871	Y: 4265714
Mundo	Cola embalse Talave	-	MU03	-	-	520	X: 592997	Y: 4263059
Mundo	Bajo presa Talave	-	MU04	-	-	480	X: 599968	Y: 4262223
Mundo	Puente romano	-	MU05	-	-	460	X: 606025	Y: 4258341
Mundo	Presa San Diego	-	MU06	-	-	430	X: 607949	Y: 4257438
Mundo	Presa Tavizna	10	MU07	-	-	396	X: 609885	Y: 4254743
Mundo	Cola embalse Camarillas	11	MU08	-	-	354	X: 618484	Y: 4250924
Mundo	Bajo presa Camarillas	-	MU09	-	-	350	X: 616863	Y: 4242227
Segura	Aguas abajo Anchuricas	-	SE01	-	-	860	X: 543949	Y: 4231159
Segura	Camping	3	SE02	-	-	685	X: 551513	Y: 4238978
Segura	Cola embalse Fuensanta	-	SE03	-	-	491	X: 560092	Y: 4242807
Segura	Peñarrubia	4	SE04	-	-	470	X: 571837	Y: 4250622
Segura	El Gallego	5	SE05	-	-	452	X: 586737	Y: 4251911
Segura	Puente Híjar	-	SE06	-	-	432	X: 593416	Y: 4250112
Segura	Bajo presa Cenajo	6	SE07	-	-	363	X: 607382	Y: 4248560
Segura	El Hondón	-	SE08	-	-	325	X: 612390	Y: 4247179
Segura	Las Minas	7	SE09	-	-	306	X: 614705	Y: 4243932
Segura	Cañaverosa	12	SE10	-	-	290	X: 612361	Y: 4237775
Segura	Postrasvase	-	-	T1	VS	268	X: 613788	Y: 4235661
Segura	Esparragal	13	SE11	T2	-	259	X: 613787	Y: 4235662
Segura	Hoya García	14	SE12	N1	-	200	X: 627272	Y: 4233623
Segura	El Menjú	-	-	N2	-	160	X: 639119	Y: 4231252
Segura	El Jarral	15	SE13	T3	SNBO	148	X: 640577	Y: 4229308
Segura	Ulea	16	SE14	-	-	112	X: 647068	Y: 4222775
Taibilla	Las Claras	2	TAI	-	-	640	X: 568287	Y: 4242241
Tus	Rala	1	TUS	-	-	809	X: 559633	Y: 4251670