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Biological Response of Invasive Fish in a Highly Regulated Mediterranean River Basin

Respuesta Biológica de Peces Invasores en una Cuenca Mediterránea Altamente Regulada

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Facultad de Biología
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Biological response of invasive fish in a highly regulated Mediterranean river basin

Respuesta biológica de peces invasores en una cuenca mediterránea altamente regulada

Memoria presentada para optar al grado de Doctor en Biología por la licenciada

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Structure of the doctoral thesis report

No specific rules to build the thesis report exist. The most general structure style is that followed by research articles, but including more original data from the study. Consequently, the more conventional structure used is the one with the follow sections: Introduction, Material and methods, Results, Discussion and Conclusions.

The present doctoral thesis report is organized modifying the conventional report structure. The two first chapters bring us closer to the problem tackled in this study and their protagonists (both the Segura River basin and the target species). The following chapters are homogeneous entities and specific results can be extracting from them, which allow an autonomous discussion. However, they are not independent in relation with the main objectives and provide complement information to achieve principal purpose of the doctoral thesis. A possible negative facet of this type of structure is the replay of some sections such as material and methods or discussion, but the Chapter 2 tries to resolve this problem.

All works of this thesis report have been developed in the research line *Biología y Conservación de Vertebrados Acuáticos* of research group *Zoología Básica y Aplicada a la Gestión y a la Conservación* in the University of Murcia. This study was financially supported by Fundación Séneca, Región de Murcia (Spain), (Project 08728/PI/08) *Efecto de la modificación antrópica del régimen hídrico sobre los peces epicontinentales nativos y exóticos de la cuenca del rio Segura* and LIFE+ Segura Riverlink (Project LIFE12 ENV/1140). Moreover, this thesis has been developed within the framework of *Programa Nacional de Formación del Profesorado Universitario* granted by the Spanish Ministry of Education and Science.

Resumen

Las invasiones biológicas se consideran una de las principales amenazas para la biodiversidad global. Los peces invasores pueden alterar la biodiversidad por medio de numeross mecanismos, como la depredación, la competencia, la hibridación, la alteración del hábitat y la transmisión de nuevas enfermedades. Además, también son responsables de la alteración de las comunidades acuáticas al causar la disminución, el desplazamiento y la desaparición de especies nativas. Los ecosistemas acuáticos y sus comunidades nativas son particularmente sensibles a las especies invasoras y aumentar el conocimiento sobre la plasticidad fenotípica de estas especies, involucrada en el proceso de adaptación a nuevas condiciones locales, es necesario para el desarrollo de programas de manejo y control.

El régimen natural de caudales propio de las cuencas Mediterráneas juega un importante papel en la estructura acuática ambiental ya que, entre otros aspectos, su magnitud y variabilidad determinan la distribución y composición de las comunidades de peces. En este contexto, la alteración de los regímenes hidrológicos naturales debido a la gestión que se hace del agua de los ríos, principalmente con criterios de demanda agrícola, junto a la creación de nuevos hábitats lénticos relacionados con las infraestructuras de regulación del caudal (ej. Embalses, Azudes, etc.), están facilitando la colonización y expansión de peces no nativos. La cuenca del río Segura se localiza en una de las zonas más áridas de la Península Ibérica y presenta un régimen hidrológico natural marcado por fuertes fluctuaciones estacionales y una aridez extrema en el periodo seco. A su vez, está considerada una de las cuencas mediterráneas más intensamente reguladas de Europa, con más de 30 presas de gran calibre, siendo los requerimientos agrícolas responsables del 90% de las demandas de agua que, junto con los cambios de usos del paisaje, constituyen la principal presión antrópica.

Las especies exóticas objetivo de este trabajo son *Gobio lozanoi* Doadrio & Madeira 2004, *Lepomis gibbossus* (L., 1758) y *Alburnus alburnus* (L., 1758), las cuales están ampliamente distribuidas en la cuenca del río Segura siendo las de mayor presencia en los sectores fluviales. *Gobio lozanoi,* aunque con cierta controversia, es considerada nativa de varias cuencas ibéricas y sus efectos como especie invasora han sido poco

estudiados. Por el contrario, los efectos negativos sobre la fauna nativa y el ecosistema acuático de *Lepomis gibbossus* y *Alburnus alburnus* están altamente contrastados pero escasamente estudiados en cuencas con un alto nivel de regulación. Estas especies han demostrado una alta capacidad de dispersión y establecimiento en ríos de la Península Ibérica, ocupando nuevos hábitats y su plasticidad biológica se ha propuesto como un factor importante implicado en su éxito invasor. Si bien, todavía es necesario aumentar el conocimiento sobre su biología y ecología en ambientes mediterráneos. Esta información debe ser esencial para el establecimiento de criterios científicos en los planes de manejo y control de especies invasoras.

La presente tesis doctoral pretende aumentar el conocimiento sobre la plasticidad poblacional de peces invasores en cuencas hidrológicas altamente reguladas del contexto mediterráneo. Para ello se han seleccionado las tres especies aludidas anteriormente y se han establecido los siguientes objetivos principales:

a) El estudio de su variabilidad intraespecífica de sus características poblacionales tales como la abundancia relativa, la estructura de la población, las tasas de crecimiento, la inversión y tácticas reproductivas y el estado condición de los individuos.

b) La determinación de las relaciones presentes entre las características poblacionales y las variables ambientales, principalmente evaluando su variabilidad a lo largo de los gradientes longitudinales y evaluando los efectos de la regulación del caudal a escala de cuenca fluvial.

Para facilitar la exposición de los trabajos realizados en la presente tesis doctoral, ésta se ha organizado en cuatro capítulos que conforman una entidad homogénea, pero no independiente en relación a la finalidad de la tesis.

Colonización y plasticidad de rasgos poblacionales de *Alburnus alburnus* (L. 1758) a lo largo del gradiente longitudinal del río en una cuenca mediterránea.

La identificación de las características más relevantes del hábitat necesarias para el éxito de invasores potenciales, como *A. alburnus* (L.), es fundamental para comprender su proceso invasivo y, por lo tanto, para diseñar programas de control efectivos. En un intento de aumentar la comprensión sobre las limitaciones ambientales presentes en la invasión de *A. alburnus*, este estudio proporciona nuevos conocimientos sobre el tiempo de residencia y la variación de las características poblacionales de esta especie a lo largo de un gradiente longitudinal en una de las cuencas hidrográficas más reguladas de la Península Ibérica.

Se muestrearon 25 localidades (tres veces en cinco años) a lo largo de la cuenca del río Segura (SE España) mediante pesca eléctrica entre los años 2005 y 2010. Para el estudio de las características poblacionales se capturaron individuos de 10 localidades durante el otoño de 2010. Un total de 1.285 individuos fueron medidos *in situ* y una submuestra de 474 fue procesada para obtener las masas total, eviscerada y las masas del hígado y las gónadas. Se estudió la abundancia, la estructura de edades y tallas, la tasa de crecimiento, la condición somática, hepatosomática y la inversión reproductora de las poblaciones. Mediante análisis de covarianza se obtuvieron los índices organosomáticos y la estructura de edades se calculó a través de longitudes retrocalculadas. Se analizó la variabilidad de las características poblacionales en relación a las variables ambientales a través de modelos de regresión.

Los datos de presencia mostraron que esta especie se ha dispersado rápidamente y está presente en más de 168 km de los tramos fluviales estudiados (84,4%). Los individuos capturados tenían una longevidad máxima de 4 años, si bien, individuos más longevos y las cohortes de individuos maduros fueron más comunes en sectores fluviales donde la especie presentaba un mayor tiempo de residencia. Los valores más altos de abundancia se dieron en la parte alta de la cuenca y se acompañaron de mayores tasas de crecimiento y tamaños máximos. Se observaron algunas tendencias de los rasgos poblacionales de la especie a nivel de cuenca, no obstante, sus relaciones con los gradientes espaciales, la respuesta específica de la especie y las diferentes etapas de invasión relacionadas con el tiempo de residencia de las poblaciones, han hecho de la interpretación una tarea difícil.

Los resultados obtenidos apoyan la hipótesis de que la amplia plasticidad poblacional de *A. alburnus* juega un papel importante en su éxito invasor en una cuenca mediterránea altamente regulada, donde este mecanismo le permite sobrevivir a los eventos de regulación de caudal a varias escalas, así como para resistir el estrés ambiental a largo plazo típico en los ríos de tipo mediterráneo.

Evaluación de los efectos de la regulación de caudal sobre variables relacionadas con el tamaño de los peces en una cuenca mediterránea.

El régimen hidrológico natural juega un papel importante en la estructura de los sistemas acuáticos y afectan al desarrollo de las poblaciones de peces. Las estructuras de regulación alteran los regímenes hidrológicos y disminuyen las perturbaciones naturales, reduciendo o eliminando la estacionalidad del caudal. Además, alteran la morfología de los ríos, generando hábitats artificiales normalmente de carácter léntico, que suponen nuevos nichos para especies más generalistas. La estructura de tamaño de las poblaciones de peces ha sido reconocida como un indicador de salud del ecosistema y refleja el efecto de la perturbación antrópica. El objetivo principal de este estudio fue evaluar el efecto de la regulación sobre la estructura del tamaño de poblaciones de peces de diferentes gremios ecológicos a lo largo del gradiente longitudinal en una cuenca mediterránea altamente regulada.

Los individuos fueron capturados mediante pesca eléctrica durante el otoño de 2010 en 29 localidades de muestreo distribuidas en 8 sectores hidrológicos con diferentes patrones de regulación. Las localidades de muestreo se clasificaron en tres tipos de tramos fluviales: (1) hábitats de caudal libre, (2) hábitats artificiales asociados a un azud de derivación y (3) hábitats artificiales lénticos. Un total de 4.825 individuos (1.560 *L. sclateri*, 1.280 *G. lozanoi*, 1.636 *A. alburnus* y 349 *L. gibbosus*) se midieron y pesaron *in situ* para obtener la longitud furcal y la masa corporal total. Se estudió la estructura de población, la condición somática y 11 variables relacionadas con el tamaño en cada especie. Se realizó un análisis de covarianza para evaluar el comportamiento de estas variables en relación con diferentes patrones de regulación (sectores hidrológicos) y con los tipos de tramo fluvial. Las localidades de muestreo se ordenaron según variables ambientales y de caudal mediante análisis de componentes principales.

Los diferentes regímenes de caudal y la presencia de hábitats artificiales derivados de estructuras de regulación influyeron sobre el estado de los individuos y la estructura de tamaño de las especies objetivo. Las respuestas observadas fueron específicas de cada especie de acuerdo con sus gremios ecológicos. El régimen de caudal natural típico de las cuencas mediterráneas resultó menos adecuado para especies típicas de entornos estables (hábitats lenticos). No obstante, la presencia de estructuras de regulación parece proporcionar entornos más estables y posibles refugios para los individuos de dichas especies.

Estrategia reproductiva y movilización de energía en *Gobio lozanoi* Doadrio & Madeira, 2004 en una cuenca mediterránea invadida.

Los rasgos reproductivos de las especies son modulados por las condiciones ambientales circundantes para lograr una estrategia de reproducción exitosa. Las especies invasoras muestran una serie de características biológicas más generalistas y presentan mecanismos para sobrevivir y adaptarse a nuevos hábitats. En relación con la asignación de nutrientes y energía en la reproducción, se definen dos grandes estrategias: *capital breeding* e *income breeding*, si bien, las especies pueden desarrollar una gama de rasgos intermedios entre ambas estrategias. El objetivo principal del presente estudio fue analizar los rasgos reproductivos y la estrategia de balance energético, durante la fase de reproducción, de *G. lozanoi* en una cuenca mediterránea invadida del sureste de la Península Ibérica.

Mediante pesca eléctrica se recolectaron peces durante un año (2015) en cinco localidades de muestreo ubicadas en sectores fluviales con diferentes regímenes hidrológicos. Se capturaron un total de 2.333 individuos y se midió la longitud furcal, masa total y eviscerada y las masas del hígado y gónada de una submuestra de 1.982 ejemplares. Se seleccionó una submuestra de 133 individuos maduros para estudiar la fecundidad, el tamaño de los ovocitos y el contenido de proteínas y lípidos en tejidos (músculo, hígado y gónada). Mediante análisis de covarianza se obtuvieron los índices organosomáticos, las diferencias entre sexos se evaluaron mediante la prueba t de Student, se realizaron análisis de varianza para determinar diferencias entre las etapas reproductivas y se estimó el tamaño de la primera madurez mediante regresiones logísticas.

La actividad gonadal fue similar para ambos sexos y se describieron cuatro etapas reproductivas en el ciclo de reproducción: inactividad, maduración, desove y postdesove. La condición somática en los machos fue más alta que en las hembras, lo que sugiere una mayor inversión energética para las hembras durante la reproducción. La especie alcanzó la madurez sexual a edades tempranas y mostró baja fecundidad y grandes ovocitos en comparación con otras poblaciones ibéricas no nativas. Los contenidos de proteínas y lípidos mostraron diferentes dinámicas en los tejidos durante el ciclo reproductivo y los resultados sugieren que *G. lozanoi* podría exhibir una estrategia de movilización energética intermedia entre *income* y *capital breeding*.

¿La regulación del caudal modula los rasgos reproductivos y la asignación de energía en la especie no nativa *Gobio lozanoi* Doadrio & Madeira, 2004 en una cuenca mediterránea?

La regulación de caudal representa la alteración antrópica más extendida en los ecosistemas acuáticos y tiene un papel importante en el desarrollo del hábitat, la disponibilidad de fuentes de alimento y la distribución de los organismos. Además, se ha demostrado que el caudal tiene un efecto significativo sobre el desarrollo de las características reproductivas. El objetivo principal de este estudio fue evaluar el efecto de la regulación del caudal en los rasgos reproductivos y la asignación de energía durante el ciclo reproductivo de *G. lozanoi* en una cuenca mediterránea invadida del sudeste de la Península Ibérica.

Durante un año (2015), se capturó un total de 2.333 individuos mediante pesca eléctrica en cinco sectores fluviales con diferentes regímenes hidrológicos. Los índices organosomáticos se evaluaron a partir de una submuestra de 1.982 individuos y se estudio la fecundidad, el tamaño de los ovocitos y el contenido de proteínas y lípidos en tejidos (músculo, hígado y gónada) en 133 individuos maduros. Los índices organosomáticos se obtuvieron mediante análisis de covarianza. Para probar las diferencias entre los sitios de muestreo por etapas reproductivas, se emplearon análisis de varianza en ambos sexos por separado.

Se observaron diferencias intraespecíficas en la dinámica temporal del desarrollo de las gónadas relacionadas con los cambios en los regímenes de caudal, mostrando un retraso en la maduración en poblaciones que habitan sectores hidrológicos donde no hay picos de caudal o éstos aparecen más tarde. La variación temporal en la condición de los peces también parece verse afectada por diferentes regímenes de caudal, manifestando valores más bajos en sectores donde los caudales altos coinciden con los eventos reproductivos. Además, en estos sectores también se observaron bajas fecundidades, ovocitos más pequeños y los individuos alcanzaron la madurez sexual a una edad más temprana, lo que sugiere un importante efecto negativo sobre el éxito reproductivo. Las poblaciones mostraron características intermedias entre la estrategia oportunista y periódica, pero en desacuerdo con la hipótesis planteada, sugiriendo que el efecto de regulación del caudal no es suficiente para cambiar la estrategia reproductiva a escala del sector hidrológico.

Conclusiones generales

Sobre la base de los trabajos presentados en esta tesis doctoral focalizada en el estudio de la plasticidad poblacional de los peces invasores más ampliamente distribuidos en sectores fluviales de la cuenca del río Segura, *Alburnus alburnus*, *Lepomis gibbosus* y *Gobio lozanoi*, se pudieron extraer las siguientes conclusiones generales:

En relación con la colonización y la plasticidad poblacional de *Alburnus alburnus* a lo largo de gradientes longitudinales de ríos:

1. La amplia variabilidad fenotípica presente en sus características poblacionales le permite adaptarse y establecer nuevas poblaciones en ríos de tipo mediterráneo altamente regulados y parece ser un factor importante en su proceso de invasión.

2. *Alburnus alburnus* está ampliamente establecido en la cuenca del río Segura y su elevada presencia confirma la viabilidad de sus poblaciones en tramos fluviales, los cuales son hábitats subóptimos de acuerdo con sus requerimientos ecológicos. Además, la regulación del caudal y la presencia de hábitats lénticos relacionados con las estructuras de regulación parecen estar afectando el estado de sus poblaciones.

3. La estructura de tamaño y edad de sus poblaciones fue similar a la observada en otras poblaciones ibéricas, mostrando menores tallas que las presentes en poblaciones nativas de la especie. Las cohortes de mayor tamaño y los peces más longevos se localizaron en los sectores fluviales donde el tiempo de residencia en la cuenca fue más extenso, mientras que la ausencia de individuos mayores coincide con etapas más tempranas del proceso invasivo, donde los peces más grandes, probablemente después de un alto esfuerzo reproductivo, podrían estar sufriendo tasas de mortalidad más elevadas.

4. La abundancia, longitud máxima y crecimiento en poblaciones de *A. alburnus* presentaron una relación negativa con el gradiente longitudinal, mostraron valores más bajos en sectores con mayor estrés ambiental, mayor temperatura y conductividad del agua, y peor estado ecológico. Además, se ha demostrado que la regulación del caudal es un factor importante que influye en las características poblacionales; de hecho, se detectaron poblaciones más abundantes y con un crecimiento más rápido en sectores fluviales con una variabilidad de caudal media y un caudal diario medio.

En relación con los efectos de la regulación del caudal sobre la estructura del tamaño de la población de los peces invasores objetivo:

5. Los diferentes regímenes de caudal y la presencia de hábitats fluviales artificiales derivados de estructuras de regulación, los cuales amortiguan los caudales drásticos naturales y proporcionan posibles refugios para peces, han tenido efectos sobre el estado de los individuos y la estructura de talla en las poblaciones de las especies objetivo. Las respuestas observadas fueron específicas de las especies de acuerdo con sus gremios ecológicos.

6. Los regímenes de caudal natural típicos de las cuencas mediterráneas y la presencia de picos de caudal derivados de las descargas de agua de los embalses fueron factores importantes que configuraron las estructuras de talla. Los peces invasores limnófilos o suprapelágicos, como *L. gibbosus* y *A. alburnus*, que no están adaptados para aguantar y superar las inundaciones repentinas estacionales, probablemente tengan más problemas para establecer poblaciones. Sin embargo, la homogeneización de los regímenes de caudal junto con la presencia de hábitats artificiales derivados de estructuras de regulación, podría favorecer su establecimiento en cohabitación con peces reófilos de agua dulce, ya sean éstos autóctonos o nativos.

7. La estructura de tamaño observada en *A. alburnus* respalda su viabilidad poblacional en sectores fluviales alejados de hábitats lénticos de los embalses. Aunque su tasa de crecimiento estuvo relacionada con la variabilidad del caudal, no se detectó un efecto significativo de la regulación del caudal sobre las variables relacionadas con el tamaño de sus poblaciones. Sin embargo, las poblaciones ubicadas junto a hábitats artificiales derivados de la estructuras de regulación de caudal dentro de los mismos sectores hidrológicos mostraron una condición somática más alta.

8. La reducción de la estacionalidad del caudal y la presencia de hábitats artificiales de caudal parecen ser factores decisivos para la viabilidad de las poblaciones de *L. gibbosus* dentro del mismo sector hidrológico. De hecho, las poblaciones más cercanas a los hábitats artificiales mostraron poblaciones con una mayor diversidad de tamaños y una mayor presencia de individuos pequeños.

9. Los lugares más alejados de los tramos fluviales con hábitats artificiales derivados de estructuras de regulación de caudal fueron tramos más adecuados para el reófilo *G. lozanoi*, mostrando una estructura de tamaño con una buena representación de todas las clases de tamaño con predominio de individuos medianos y grandes. Sin embargo, los tramos más cercanos a zonas lénticas derivadas de la presencia de barreras longitudinales (ej. presas, azudes, etc.) parecen ser más apropiadas para la supervivencia de individuos más pequeños, tal como ocurrió también con el pez nativo reófilo *L. sclateri*.

En relación a los rasgos reproductivos y la estrategia de balance energético de *Gobio lozanoi* que habitan diferentes sectores fluviales:

10. Los rasgos reproductivos de *G. lozanoi* en la cuenca del río Segura mostraron cierto grado de diferencia con otras poblaciones no nativas de la Península Ibérica. Sin embargo, una maduración temprana y un periodo de desove prolongado concuerdan con la estrategia reproductiva desarrollada en dichas poblaciones ibéricas, siendo características típicas de especies que muestran una estrategia del tipo *income breeding*. Sin embargo, la asignación de energía observada en nuestro estudio reveló datos que indican la presencia de una estrategia intermedia en la especie que se comportó más como un *income-capital breeder*. Además, los regímenes de caudal tuvieron efectos significativos sobre los rasgos reproductivos, sin embargo, no se puede concluir acerca de cambios en la estrategia de asignación de energía entre los sectores hidrológicos fluviales con diferentes regímenes de caudal.

11. Se describieron cuatro etapas reproductivas (quiescencia, maduración, desove y postdesove) en el ciclo reproductor, mostrando fases de maduración y desove más largas que en otras poblaciones ibéricas no nativas. La condición de las poblaciones (somática y hepatosomática) mostró una inversión sexual diferente con un mayor coste energético para las hembras durante la reproducción y también una mayor actividad hepática relacionada con el proceso de vitelogénesis durante la maduración y el desove.

12. Las poblaciones estudiadas mostraron un tamaño menor de primera madurez, menor fecundidad y ovocitos más grandes que otras poblaciones ibéricas no nativas. Debido a que el tiempo de colonización de la cuenca por la especie es muy largo, estas diferencias podrían estar más relacionadas con una estrategia de adaptación a las características de los factores ambientales de una cuenca mediterránea altamente regulada que relacionada con su proceso de invasión.

13. La estabilidad del contenido de lípidos en el hígado y las gónadas para ambos sexos y el incremento de proteínas ováricas y hepáticas en las hembras sugieren que la mayor parte del aporte energético durante la reproducción proviene de la alimentación *in situ* de los individuos, que es típica de las especies del tipo *income breeders*. Sin embargo, la disminución de los lípidos en el músculo durante la maduración y el desove podría mostrar un cierto grado de movilización de energía desde los tejidos musculares, que está más relacionado con una estrategia del tipo *capital breeding*.

14. Se observaron diferencias intraespecíficas en la dinámica temporal del desarrollo de las gónadas relacionadas con los cambios en los regímenes de caudal, que muestran una maduración retrasada en las poblaciones que habitan los sectores hidrológicos donde no hay picos de caudal o éstos aparecen más tarde. La variación temporal en la condición de los peces también parece verse afectada por diferentes regímenes de caudal, mostrando valores más bajos en los sectores donde los caudales altos coinciden con el evento reproductivo.

15. La proporción de sexos se inclinó hacia las hembras, excepto en los sectores hidrológicos con un régimen de caudal inverso, caracterizado por un alto nivel de caudal en los períodos secos de verano y la pérdida de inundaciones repentinas, lo que podrían causar una alta mortalidad de las hembras debido a la dura inversión reproductiva. En este sector, los individuos alcanzaron la madurez sexual a una edad temprana, se registró una menor fecundidad y los ovocitos más pequeños, lo que sugiere un importante efecto negativo sobre el éxito reproductivo.

16. Las poblaciones mostraron características intermedias entre la estrategia de vida oportunista y periódica y los diferentes regímenes de caudal parecen modular diferencias intraespecíficas. Sin embargo, en desacuerdo con la hipótesis propuesta, los resultados sugieren que los efectos de regulación de caudal no son lo suficientemente fuertes como para cambiar la estrategia reproductiva a escala de sector hidrológico.

Chapter 1

General introduction and objectives

Introduction

Biological invasions are considered one of the main threats to global biodiversity (Clavero & García-Berthou, 2005; Early *et al.*, 2016). Invasive alien species, defined as those non-native species that threaten ecosystems, habitats and species (Convention on Biological Diversity, 2008) are considered as important drivers of the ongoing global environmental change (Pyšek & Richardson, 2010). In fact, they can alter biodiversity by means of several mechanisms, such as predation, competition, hybridisation, habitat alteration and transmission of new diseases (Cucherousset & Olden, 2011; Powell *et al.*, 2011). Generally, ranked as the second greatest cause of species endangerment and extinction after habitat destruction (IUCN, 2011), invasive species can also seriously affect ecosystem services that are fundamental to human survival and well-being (Millennium Ecosystem Assessment, 2005; Vilà *et al.*, 2010).

Freshwater ecosystems and their native communities are particularly sensitive to invasive species (Clavero, 2011). In a global perspective, invasive fish have been reported to alter aquatic communities by causing the decline, displacement and disappearance of native species (Gozlan *et al.*, 2010; Cucherousset & Olden, 2011). They are also responsible for abundance and distributional range reductions, as well as local or global extinction of many native fish species worldwide (Leunda, 2010; Ellender & Weyl, 2014; Clavero & García-Berthou, 2005; Gallardo *et al.*, 2016). Additionally, it has been suggested that increased habitat destruction related to human alteration has favoured the establishment and success of invasive exotic species, resulting in a subsequent loss of biodiversity (Hermoso *et al.*, 2011; Jackson *et al.*, 2001; Malmqvist & Rundle, 2002).

One of the key factors for the conservation of global biodiversity is the management of such invasive species, with the specific objective of eliminating or reducing the negative impacts that they produce (Meffe & Carroll, 1997; Helfman, 2007; Clout & Williams, 2009). In relation with freshwater fish, increased knowledge of their high phenotypic plasticity involved in the adaptation of invasive species to local conditions is necessary for management and control programs (Britton *et al.*, 2011). Moreover, invasion success of fish introduced outside their native range is often explained by the expression of their life history traits that are also strongly influenced by environmental conditions (Vila-Gispert *et al.*, 2005; Ribeiro & Collares-Pereira, 2010). Considerable intraspecific plasticity is often observed in the process of adaptation to a new environment (García-Berthou, 2007; Ribeiro *et al.*, 2008), and life history variability seems to play an important role in the invasion success (Ribeiro & Collares-Pereira, 2010; Ruiz-Navarro *et al.*, 2013; Almeida *et al.*, 2014; Bergerot *et al.*, 2015). In the Iberian Peninsula, and for decades, biological plasticity has been proposed as an important factor for understanding the invasive success of several invasive freshwater fishes (Fernández-Delgado, 1990; Fernández-Delgado & Rossomanno, 1997; Vila-Gispert *et al.*, 2005: Ribeiro & Collares-Pereira, 2010; Ruiz-Navarro *et al.*, 2011; Almeida *et al.*, 2014; among others). However, there is a clear need for information on population traits of several species in different invaded areas (García-Berthou, 2007; Riberio *et al.*, 2009; Leunda, 2010; Hermoso *et al.*, 2011).

Natural hydrological regime plays an important role on the environmental aquatic structure (Poff, 1997; Lytle & Poff, 2004). Magnitude and variability of flow regime results a strong driving force on the evolution and distribution of fish fauna inhabiting particular fluvial areas (Langerhans & Reznik 2009; Weiher *et al.*, 2011; Gutiérrez-Cánovas *et al.*, 2015; Bennett & Kozak, 2015). In fact, although Mediterranean flow regime is considered one of the main factors to control the responses of fish populations (Oliva-Paterna *et al.*, 2003; Bernardo *et al.*, 2003; Vila-Gispert *et al.*, 2005), the loss of natural regimes, together with the creation of lentic habitats related to flow regulation infrastructures (e.g. reservoirs and weirs) are encouraging the establishment of non-native fishes (Bunn & Arthington, 2002; Alexandre & Almeida, 2010; Ribeiro & Collares-Pereira, 2010; Clavero *et al.*, 2013; Almeida & Grossman, 2014). In spite of all this, there are scarce works that enquire into the biology of exotic fish and their intraspecific variability in relation with effects of flows (Ribeiro *et al.*, 2008; Liermann *et al.*, 2012; Almeida *et al.*, 2014).

In this context, the present PhD thesis aims to provide basic information about the population plasticity in biological traits of invasive exotic fishes inhabiting a high regulated Meditarranean basin in the south-eastern Iberian Peninsula. This information could be essential for the establishment of scientific criteria in management and control programs of invasive species. To achieve it, the following general objective was proposed:

To increase the knowledge about intraespecific plasticity of the most widely distributed invasive fish in fluvial sectors of the Segura River basin, *Alburnus alburnus* (L., 1758), *Lepomis gibossus* (L., 1758) and *Gobio lozanoi* Doadrio & Madeira, 2004.

a) To study intraespecific variability of the population traits such as relative abundance, population size structure, growth rates, reproductive investment and tactics, and fish status condition.
b) To determine relationships between their population traits and environmental features, mainly assessing their variability throughout the longitudinal gradients and evaluating flow regulation effects at a river basin scale.

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Chapter 2

Approximation to study area and target species

Study area

The Segura River basin is located in the southeast of the Iberian Peninsula and has an area of 18.870 km^2 (CHS, 2007). The basin represents 3.81% of the national territory and comprises part of the provinces of Albacete, Alicante, Almeria, Jaén, Granada and the most of Murcia (CHS, 2007). The basin is formed by two main longitudinal axes, the Segura River and the Mundo River. The Segura River has an approximate length of 325 km and the Mundo River, its main tributary, runs for about 119 km before flowing into the Segura River.

The basin is located in a Mediterranean climate area and it is characterised by high aridity, and it has a particular geomorphology due to its geology, lithology and intense erosive processes (Vidal-Abarca *et al.*, 1990). It represents one of the most arid zone of the Iberian Peninsula (Vidal-Abarca *et al.*, 1987) and, probably, of Europe within the Mediterranean area (Geiger, 1973). The basin shows a strong climatic and altitudinal gradient and a pronounced hydrological variability on both spatial and temporal scales. Its hydrological regime is marked by strong seasonal natural fluctuations with flash floods in spring and autumn and severe droughts in summer (CHS, 2007). Moreover, scarce rainfall and evapotranspiration phenomena make it one of the basins with the greatest flow deficit.

The Segura River is one of the most intensively regulated Mediterranean basins in Europe (MMA, 2004), where irrigation is responsible for the 90% of water demands and constitutes the main anthropogenic pressure on stream flows, followed by urban uses and industrial and environmental requirements with a 7% and 2% of water demands, respectively (Grindlay *et al.*, 2011). Flood control and water demand have been the main reasons promoting dams building in this region. Currently, to be able to take on this great water demands, the basin shows 33 large dams (more than 15 m height) and 121 weirs higher than 2 m (CHS, 2007). Also, since the 1980s an inter-basin water transfer has operated from the Tagus River to the Mundo River (Grindlay *et al.*, 2011). The major reservoirs present in the basin are Fuensanta and Cenajo with 210 hm^3 and 437 $hm^3$of capacity respectively, which are located in the Segura River, and Talave (35 hm^3) (which receives the waters from the Tajo-Segura Transfer) and Camarillas reservoirs (36 hm^3) located in the Mundo River (CHS, 2007). Under these circumstances, the natural flow regime is highly altered, with a decrement of flow magnitudes and a reverse seasonal flow pattern in some areas (Vidal-Abarca *et al.*, 1990; Belmar-Díaz *et al.*, 2011).

The fish assemblage in the Segura River basin has suffered a great change in the last 25 years (Oliva-Paterna *et al.*, 2014). Currently, it is composed of both native and non-native species, but introduced species are abundant and widely distributed in the basin (Andreu-Soler *et al.*, 2006; Martínez-Morales *et al.*, 2010). The native southern Iberian barbel, *Luciobarbus sclateri* (Günther, 1868), is the most frequent species in whole study area, followed by the allochthonous non-native species Pyrenean gudgeon, *Gobio lozanoi* Doadrio & Madeira, 2004. Native species brown trout *Salmo trutta* L., 1758 and southern Iberian chub *Squalius pyrenaicus* (Günther, 1868) and the allochthonous species southern Iberian spined-loach *Cobitis paludica* (de Buen, 1930) are only present in upper reaches (Oliva-Paterna *et al.*, 2014). In the middle reaches and associated to reservoirs, non-native ichthyophagous species such as northern pike *Esox lucius* L., 1758, pike-perch *Sander lucioperca* (L., 1758) and largemouth black bass *Micropterus salmoides* (Lacepèd, 1802) are present. Other non-native species present in the basin are common carp *Cyprinus carpio* L., 1758, goldfish *Carassius auratus* (L., 1758), rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792), pumpkinseed fish *Lepomis gibbosus* (L., 1758), bleak *Alburnus alburnus* (L., 1758) and the translocated Iberian straight-mouth nase *Pseudochondrostoma polylepis* (Steindachner, 1864).

In this study, the sampling area is located in the upper and middle part of the Segura River basin. The total number of sampling sites was 29 and they were distributed along the two main longitudinal axes, the Segura River (SE0-SE17) and the Mundo River (MU0-MU8) and two small headwater tributaries Taibilla (TAI) and Tus (TUS) rivers (Fig. 2.1). Depending on the study carried out in each chapter, the number of sampling sites was different.

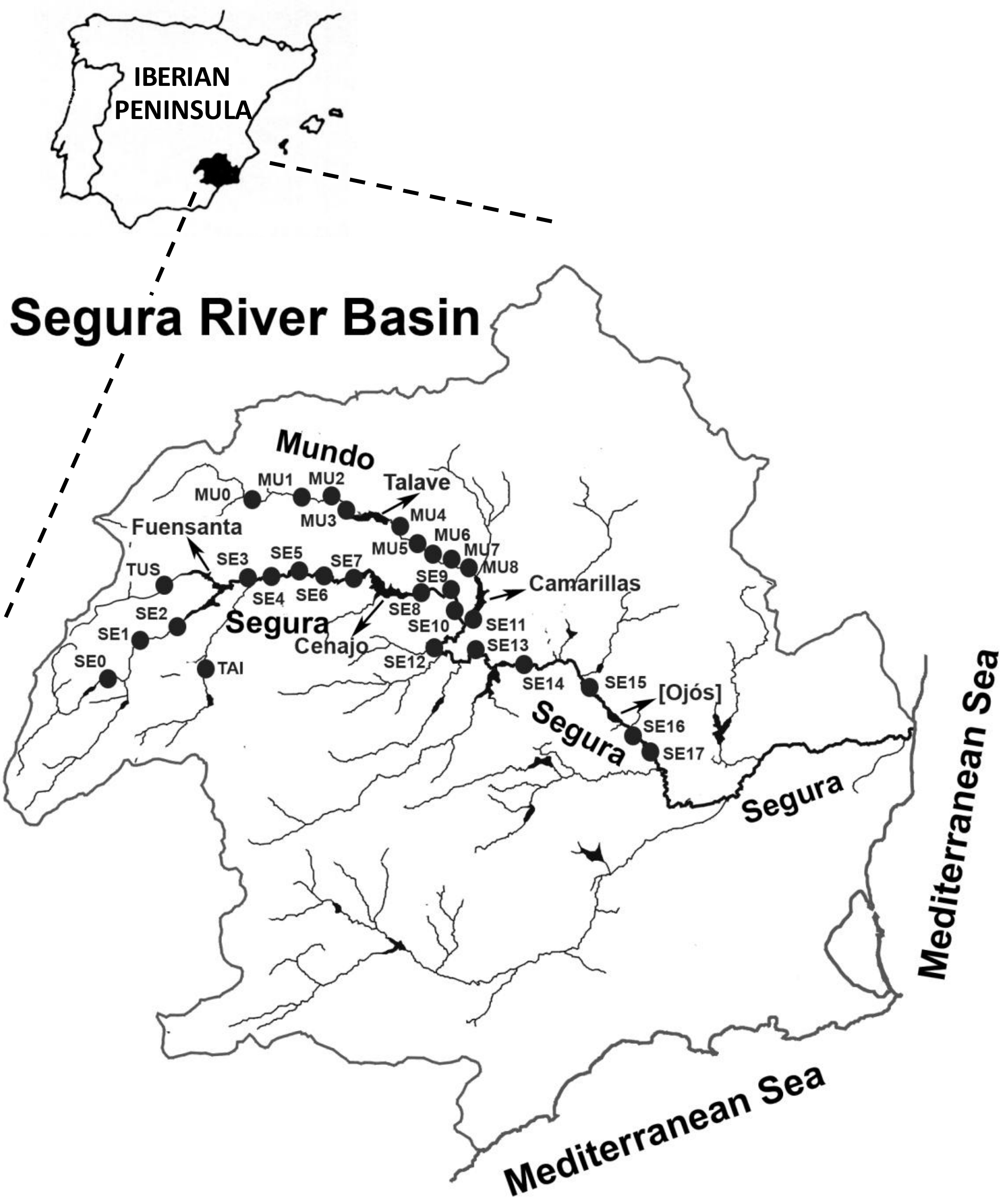

Figure 2.1. Segura River basin. Sampling sites located in Segura River (SE0-SE17), in Mundo River (MU0-MU8) and tributaries (TAI and TUS). Principal reservoirs are indicated (arrows).

Flow characterisation

Presence of flow regulation structures (large dams and weirs) has impacts on the hydrology of the rivers upstream and downstream at different temporal scales (daily, monthly and annually) (Piqué *et al.*, 2016). The flow regime patterns are altered affecting monthly flows, flood magnitude and frequency, diminishing natural flow disturbances, reducing or losing flow seasonality and variability downstream of dams (Bunn & Arthington, 2002; Poff *et al.*, 2007; Piqué *et al.*, 2016). Moreover, longitudinal fragmentation of rivers by artificial barriers implies changes in water quality and temperature, alter physical surrounding habitat and food availability, and finally, it modifies the natural dynamic and functionality of rivers (Richter *et al.*, 1996; Vörömarty *et al.*, 2003).

Hydrological sector characterisation in the study area was carried out using mean daily flow data collected from gauging stations along the rivers (CHS, 2007) and converted to a standard unit of measurement (m^3 s^{-1}). Flow metrics were calculated with the Time Series Analysis TSA within the River Analysis Package (RAP version 3.0.7) (Marsh *et al.*, 2006) and the hydrological periods considered were from year 1994 to 2010 in Chapter 4 and from year 1994 to 2015 in Chapter 6.

Nine gauging stations between dams were selected to hydrological sector characterisation in both Segura and Mundo rivers (Table 2.1). Three of the gauging stations (Calasparra, Almadenes and Cieza) showed similar daily flow pattern and a posterior ordination analysis (see Principal Component Analysis in Chapter 4) grouped them together. For this reason, they were considered as the same hydrological sector (Table 2.1). No flow data were available from tributary rivers Taibilla and Tus, and the data from upper part of Mundo River were used.

Table 2.1. Gauging stations in which flow data have been recorded. Sampling sites included in each river stretch where the gauging stations are located and hydrological sectors grouping sampling sites with the same flow characteristic.

Gauging station	Sampling site	Hydrological sector
--	TAI, TUS	HS0
Liétor	MU0, MU1, MU2, MU3	HS1
Talave	MU4, MU5, MU6, MU7, MU8	HS2
Fuensanta	SE0, SE1, SE2	HS3
Gallego	SE3, SE4, SE5, SE6, SE7	HS4
Cenajo	SE8, SE9, SE10	HS5
Calasparra	SE11, SE12, SE13	
Almadenes	SE14	HS6
Cieza	SE15	
Ojós	SE16, SE17	HS7

Exotic target species

In this study, the effect of flow on biology traits of non-native freshwater fish has been tested. The selected species are *Gobio lozanoi* Doadrio & Madeira 2004, *Lepomis gibbossus* (L., 1758) and *Alburnus alburnus* (L., 1758). They are widely distributed in the Segura River basin and have a high frequency of occurrence in the study area (Oliva-Paterna *et al.*, 2014).

Gobio lozanoi Doadrio & Madeira, 2004

The Pyrenean gudgeon, *Gobio lozanoi* (Actinopterygii, Cyprinidae), is an endemic species from the Iberian Peninsula and the south of France. Its native range includes the French rivers Adour and Nivelle and the Spanish rivers Ebro and Bidasoa (Doadrio & Madeira, 2004), but, currently, the native character of *G. lozanoi* in the Iberian Peninsula is controversial (Elvira *et al.*, 2005; Doadrio *et al.*, 2011). The species has been introduced into several Iberian catchments as live bait for angling and, nowadays, is widely distributed across the Iberian Peninsula with established populations in a large number of rivers (Doadrio *et al.*, 2011; SIBIC, 2014).

Gobio lozanoi is a small cyprinid, which is usually not larger than 15 cm in total length (Lobón-Cerviá & Torres, 1984; Lobón-Cerviá *et al.*, 1991). Population age structure of the specie varies depending on the river basin, but used to reach 5+ years (Lobón-Cerviá & Torres, 1984; Miñano *et al.*, 2003). The species reaches sexual maturity at an early age. Normally, the majority of females and males of age 2+ are mature individuals (Lobón-Cerviá & Torres, 1984; Lobón-Cerviá *et al.*, 1991; Miñano *et al.*, 2003). Respect to the reproductive cycle, gonads begin to develop at the beginning of spring and they reach their maximum around May. The spawning period involves from spring to the end of summer, but the reproductive cycle may vary spatially (Lobón-Cerviá & Torres, 1984; Lobón-Cerviá *et al.*, 1991; Miñano *et al.*, 2003). The species is a multiple spawner and no parental care is present (Lobón-Cerviá & Torres, 1984; Lobón-Cerviá *et al.*, 1991; Miñano *et al.*, 2003).

The species presents an inferior mouth and benthic habits so its diet is composed mainly of organisms present in the substrate and captures of terrestrial or superficial invertebrates are limited (Oscoz *et al.*, 2003, 2006). The more consumed preys were crustaceans (Cladocera), molluscs, insect larvae (Chironomidae, Simulidae, Ephemeridae, Baetidae, and Hidropsichidae) and invertebrates of terrestrial origin (especially Diptera), although the abundance detected depends of the different availability of preys (Oscoz *et al.*, 2003, 2006). Plant matter and sediment appeared sparsely and its intake occurs accidentally a consequence of the exploration of the substrate (Oscoz *et al.*, 2003, 2006).

The species usually inhabits middle reaches of rivers where the bottoms are sand or gravel (Doadrio, 2001), but *G. lozanoi* has demonstrated a high ability to dispersion and colonization and it has been detected in all types of environments (Doadrio & Madeira, 2004, Martínez-Morales *et al.*, 2010; Doadrio *et al.*, 2011; Amat-Trigo *et al.*, 2013). The species shows latitudinal variation in the use of habitats colonizing rivers of moderate flow, clear waters and bottoms of sand or gravel in the north of the Iberian Peninsula and rivers with slow flow and clay substrates in the central and south area (Doadrio & Madeira, 2004, Doadrio *et al.*, 2011). The species has been also found in large pools with sedimentation zones with scattered patches of aquatic vegetation (Muñoz-Mas *et al.*, 2016a) and it is the most abundantly introduced species in rivers with altered channels (Ilheu *et al.*, 2014).

In spite of the species being native to the Iberian Peninsula, some authors consider *G. lozanoi* shows a high capacity of spread and can behave a dangerous invader, rapidly increasing its density and occupying new habitats (Elvira, 2010; Doadrio *et al.*, 2011). *G. lozanoi* is cataloged as species lie between the opportunistic and periodic life strategy (Vila-Gispert & Moreno-Amich, 2002), ontogenetic variation in their diet and use of habitats provides this species the ability to establish viable populations in many Iberian basins (Grossman *et al.*, 1987; Grossman & Sostoa, 1994; Oscoz *et al.*, 2003, 2006; Amat-Trigo *et al.*, 2013). However, information about its biology is scarce and no studies dealing with their direct impacts exist. This absence of information could be hiding real negative effects of this species (Leunda, 2010; Ribeiro & Leunda, 2012), because, for example, some studies suggesting intense interspecific competition for food resources (Valladolid & Przybylski, 1996; Oscoz *et al.*, 2006) and the presence of the protozoan *Myxobolus bramae* Reuss, 1906 and the nematode *Philometra ovate* (Zeder, 1803) could indicate disease transmission to other fish (Cordero del Campillo *et al.*, 1994; Saraiva *et al.*, 2008). Other potential impact could be ecosystem alteration, as it has been demonstrated for its congener *Gobio gobio* (L., 1758), whose presence depresses the overall abundance and biomass of meiofaunal assemblages (Weber & Traunspurger, 2014).

Figure 2.2. *Gobio lozanoi*. Image from C. González Revelles©

Lepomis gibbosus (Linnaeus, 1758)

The pumkindseed fish, *Lepomis gibbosus* (Pisces, Centrarchidae) is a native fish from North America (NatureServe, 2010). It has been introduced mainly in other areas of North America and Europe, but also in South America, Asia and Africa (De Magalhães & Ratton, 2005; Cucherousset *et al.*, 2009, NatureServe, 2010). Its introduction in Europe dates from around 1880 (Vooren, 1972) and its first confirmed record in the Iberian Peninsula was in 1910 (García-Berthou & Moreno-Amich, 2000a). Nowadays, it is a successful invader in much of the Iberian Peninsula (Doadrio *et al.*, 2011) and is also one of the most common species in southern Europe (Ferreira *et al.*, 2007).

Lepomis gibbosus is high and compressed laterally with an attractive colouring olive green and a characteristic black spot, with the posterior limit orange or red, located on each operculum (Kottelat & Freyhof, 2007). In its native range, the species can reach sizes up to 40 cm total length (L_T) and present multiple age classes (generally up to 9 years) (Page & Burr, 1991), while European populations present smaller maximum sizes, around 20 cm L_T (Vila-Gispert *et al.*, 2007) and individuals rarely exceed 7 years of age (Danylchuk & Fox, 1994; Gutierrez-Estrada *et al.*, 2000; Ribeiro & Collares-Pereira, 2010). Marked sexual dimorphism does not exist; in its native range males can present a more intense coloration and larger sizes (Scott & Crossman, 1973), but in Europe no sexual differences have been observed (Gillespie & Fox, 2003; Copp & Fox, 2007).

The reproduction of *L. gibbosus* takes place between spring and summer, but its duration can vary at spatial scale, which works in favour of the invasive capacity of the species (Garvey *et al.*, 2002; Ribeiro & Collares-Pereira, 2010; Fox *et al.*, 2011). It presents multiple reproductive events within a year and paternal care carried out by territorial males (Garvey *et al.*, 2002). In its native range, the species is considered within the category of "equilibrium" according to the classification of Winemiller & Rose (1992), but in its introduced area it presents a more opportunistic strategy, with longer reproductive periods and early sexual maturation (Fox *et al.*, 2007).

The species presents a superior mouth with projectable jaws, which permits to open their mouth more and to make suction movements. It is an omnivorous species, which consumes a wide diversity of prey such as zooplankton, benthic larvae of insects, crustaceans, molluscs, fish eggs or small fish (García-Berthou & Moreno-Amich, 2000b; Andraso, 2005; Almeida *et al.*, 2009; Dextrase & Mandrak, 2006). The proportion of preys depend on their availability and *L. gibbosus* presents ontogenetic changes in the diet (Osenberg *et al.*, 1992; Zapata & Granado-Lorencio, 1993; García-Berthou & Moreno-Amich, 2000b), also linked to changes in habitat use (Vila-Gispert & Moreno-Amich, 1998).

In its native range, the species typically inhabits small lakes and ponds or shoreline of large lakes with vegetation. In the rivers it inhabits zones of low flow, clear waters, with submerged vegetation or rocky areas where it finds refuge (Scott & Crossman, 1973). Its high adaptability has allowed it to colonize different aquatic systems such as rivers, lakes, ponds and reservoirs (VilaGispert *et al.*, 2007; Almeida *et al.*, 2009; Vila-Gispert & Moreno-Amich, 1998; Copp & Fox, 2007). In the Iberian Peninsula, *L. gibbosus* is more abundant in deteriorated areas and artificial systems (such as reservoirs) (Almeida *et al.*, 2009; Matono *et al.*, 2013; De Miguel *et al.*, 2014).

Biological plasticity has been proposed as an important factor for the invasive success of *L. gibbosus*, which favours it to adapt well to new environments (Vila-Gispert *et al.*, 2005; Copp & Fox, 2007; Ribeiro & Collares-Pereira, 2010). In fact, several studies reveal how population traits of *L. gibbosus* (native or introduced populations) such us juvenile growth, sexual maturation, thermal tolerance or morphology can vary according to environmental conditions (Copp *et al.*, 2002; Fox *et al.*, 2007; Vila-Gispert *et al.*, 2007; Cucherousset *et al.*, 2009; Rooke & Fox, 2014).

The negative impact of introduced populations of *L. gibbosus* has been widely recorded and the species represents a threat to the native fauna (Clavero & García-Berthou, 2006; Dextrase & Mandrak, 2006; Leunda *et al.*, 2010; Ribeiro & Leunda, 2012). Some of the impacts generated by its introduction may be parasites transmission (Sterud & Jorgensen, 2006), habitat and trophic competence (Copp *et al.*, 2004; Copp *et al.*, 2010), aggressive behavior to native fauna (Almeida *et al.*, 2014a) or habitat alteration (increment of turbidity or nutrient levels) (Angeler *et al.*, 2002).

Figure 2.3. *Lepomis gibbosus.* Image from A. Harka (Froese & Pauly, 2018).

Alburnus alburnus (Linnaeus, 1758)

The bleak, *Alburnus alburnus* (Actinopterygii, Cyprinidae), is native from most Europe and West Asia (Freyhof & Kottelat, 2008). It has been introduced in the Iberian Peninsula, Italy, Ireland, Great Britain (except southeast), Norway, Cyprus and Algeria (Elvira, 1988; Freyhof & Kottelat, 2008; Kara, 2011). In the Iberian Peninsula it has been repeatedly introduced in reservoirs as a "forage fish" for non-native piscivorous predators (Elvira & Almodóvar, 2001) and the species has rapidly spread in freshwaters as a successful invasive species (Elvira & Almodóvar, 2001; Leunda, 2010; Almeida *et al*., 2014b).

Alburnus alburnus is a fusiform cyprinid laterally compressed and with a silvery colouration. It presents a very long base of its anal fin and a caudal fin much forked (Freyhof & Kottelat, 2008). The species can reach 25-30 cm of total length in their native areas, while in the Iberian Peninsula no fish larger than 25 cm of total length has been recorded (Pérez-Bote *et al*., 2004; Vinyoles *et al*., 2007; Almeida *et al*., 2014b). In introduced lotic areas, the species shows lower sizes, around 20 cm of total length (Almeida *et al*., 2014b). Moreover, lifespan is longer in native lakes, with individuals 9-10 years old in such populations (Bíró & Muskó, 1995; Vašek & Kubečka, 2004), than Iberian populations, which present 3-5 year classes (Masó *et al*., 2016). It is a gregarious species and it reaches maturity for the first time at 2-3 years. Its spawning period extends between May and August with several batches, but spawning duration can vary at spatial scale (Froese & Pauly, 2018). No parental care is observed.

The species presents a superior mouth, suitable to feed in the water column. It shows a daytime feeding periodicity more intense during the first part of the day and it also shows different seasonal feed intensity (Politou *et al*., 1993). The main preys were zooplankton (Cladocera, Copepoda and Harpacticida) and benthic chironomids and crustaceans, but the percentage of preys depend on their occurrence (Politou *et al*., 1993; Bíró & Muskó, 1995; Latorre *et al*., 2016). The size of fish individuals does not seem to have effect on the diet (Politou *et al*., 1993). In open waters, the species feeds at the surface, while it forages along the bottom in shallower shore areas (Haberlehner, 1988). Differences in diet composition were found at different environments (lentic-lotic) (Almeida *et al*., 2017).

In its native distribution area, the species inhabits lentic habitats such as lakes and medium to large river sectors where it can form shoals reaching high densities (Freyhof & Kottelat, 2008). Medium and large individuals are more abundant in open waters, while small fish prefer calm waters close to the shore with dense macrophytes and coves (Haberlehner, 1988). In the introduced areas, *A. alburnus* presents viable populations at diverse aquatic systems, from reservoirs to small streams (Vinyoles *et al*., 2007; Almeida *et al*., 2014b; Masó *et al*., 2016; Muñoz-Mas *et al*., 2016b) and it has been suggested that regulated rivers favour its expansion and population viability (Vinyoles *et al*., 2007; Almeida *et al*., 2014b).

Alburnus alburnus is a successful invader in much of the Iberian Peninsula (Vinyoles *et al.*, 2007), because it has several features that facilitate invasiveness and adaptability to new environments, such a high fecundity, the ability to feed on an extensive range of prey and wide temperature tolerance (Chappaz *et al.*, 1987; Latorre *et al.*, 2016). The species poses an important threat to a wide range of native fish fauna in invaded areas (Leunda, 2010). Genetic hybridation with the endemic calandino *Squalius alburnoides* (Steindachner, 1866) complex and southern Iberian chub *Squalius pyrenaicus* (Günther, 1868) threatens the genetic integrity of Iberian fishes (Almodóvar *et al.*, 2012). Moreover, trophic and habitat competition, disease transmission, predation or habitat alteration are other possible impacts which are attributed to the species (Doadrio & Angueler, 2007; Leunda, 2010; Almeida & Grossman, 2012).

Figure 2.4. *Alburnus alburnus*. Image from A. Harka (Froese & Pauly, 2018).

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Chapter 3

Colonization and plasticity in population traits of the *Alburnus alburnus* (L. 1758) along a longitudinal river gradient in a Mediterranean basin

Introduction and objectives

Biological invasions are considered one of the main threats to global biodiversity, with freshwater ecosystems and their native communities being particularly sensitive to invasive species (Clavero, 2011). Invasive fish have been reported to alter aquatic communities by causing the decline, displacement and disappearance of native species, (Cucherousset & Olden, 2011) and several studies have shown that they are a significant threat to Iberian native icthyofauna (Leunda, 2010; Hermoso *et al.*, 2011). In spite of this, many aspects related to invasive fish establishment are poorly understood.

The invasion success of fish introduced outside their native range is often explained by the expression of their life history traits that are strongly influenced by environmental conditions (Vila-Gispert *et al.*, 2005; Ribeiro & Collares-Pereira, 2010). In fact, the assessment of the growth and population structure provides ecological insights into how populations respond to abiotic and biotic factors (Ruiz-Navarro *et al.*, 2013; Bergerot *et al.*, 2015). Considerable intraspecific plasticity is often observed in the process of adaptation to a new environment (García-Berthou, 2007; Ribeiro *et al.*, 2008), and life history variability seems to play an important role in driving invasion success (Ribeiro & Collares-Pereira, 2010; Almeida *et al.*, 2014).

The bleak, *Alburnus alburnus* (Linnaeus, 1758) (Actinopterygii, Cyprinidae), is a successful invader in much of the Iberian Peninsula (Vinyoles *et al.*, 2007) and is widely distributed in Europe. Since the last three decades, it has been repeatedly introduced in reservoirs as a "forage fish" for non-native piscivorous predators (Elvira & Almodóvar, 2001) and the species has rapidly spread through Iberian freshwaters as an invasive species with potential that include competition, disease transmission, hybridization and habitat alteration (Freyhof & Kottelat, 2008; Leunda, 2010; Almodóvar *et al.*, 2012) (see Chapter 2 for more information). *Alburnus alburnus* has several features that facilitate invasiveness and adaptability to new environments, such a high fecundity, the ability to feed on an extensive range of prey and wide temperature tolerance (Chappaz *et al.*, 1987; Latorre *et al.*, 2016). Moreover, it has been suggested that the species shows higher dispersal rates in regulated rivers and there is a relationship between their expansion and population viability (Vinyoles *et al.*, 2007; Almeida *et al.*, 2014). In fact, although Mediterranean flow regimes are considered to be leading factors in controlling fish population responses, the loss of natural regimes, together with the creation of lentic habitats related to flow regulation infrastructures (i.e. reservoirs and weirs) are encouraging the establishment of non-native fishes (Ribeiro & Collares-Pereira, 2010; Clavero *et al.*, 2013). However, no studies have considered large longitudinal gradients in comparative approaches to intraspecific variations in the biological traits of *A. alburnus*, and, with the exception of a recent study which compared some of its growth and reproductive traits in five of the main Iberian watersheds (Latorre *et al.*, 2018), the studies of *A. alburnus* life history in Iberian non-native areas have mainly focused on man-made aquatic systems (Almeida *et al.*, 2014, 2017) or local scales (Masó *et al.*, 2016; Muñoz-Mas *et al.*, 2016).

Increased knowledge of the phenotypic plasticity involved in the adaptation of invasive species to local conditions is necessary for management and control programs (Britton *et al.*, 2011). Although its biological plasticity has been proposed as an important factor for understanding the invasive success of *A. alburnus* (Almeida *et al.*, 2014; Masó *et al.*, 2016), there is a clear need for information on its population traits in different invaded areas, and nothing is known about its intraspecific variability along longitudinal upstream-downstream gradients. Since the invasive success of *A. alburnus* seems to be favoured by flow regulation and the loss of natural flow regimes, the proposed hypothesis was that *A. alburnus* presents a high phenotypic plasticity which enables it to establish large populations in fluvial sectors of Mediterranean systems. The principal purpose of the present study was to evaluate population features of this species along a longitudinal gradient in a highly regulated Mediterranean stream in southeastern Spain (Segura River basin), because understanding its biology in a highly regulated basin could have important implications for management and control programs. To achieve this goal the following objectives were proposed:

a) To study intraespecific variability of the population traits of *A. alburnus* such as length-frequency distribution, abundance, growth rates, reproductive investment and organosomatic indexes in a high regulated river basin.

b) To determine relationships between population traits and environmental gradients and to assess the variability of population traits throughout the longitudinal gradients.

Material and methods

Study area

The Segura River basin (drainage area of 18,870 km^2) is an intensively regulated Mediterranean basin located in the southeast of the Iberian Peninsula. The climate of the basin is Mediterranean but with strong climatic and altitudinal gradient; the area is characterised by a pronounced hydrological variability on both spatial and temporal scales. It is one of the most intensively regulated Mediterranean basins in Europe, where irrigation is responsible for the 90% of water demands and constitutes the main anthropogenic pressure on stream flows. The regulation capacity involving dams is equivalent to over 140% of the natural inputs and since the 1980s an inter-basin water transfer has operated from the Tagus River to the Mundo River (Grindlay *et al.*, 2011). In fact, colonization of the Segura River by *A. alburnus* has been partially attributed to fish dispersion along that Tagus-Segura interbasin system (Andreu-Soler *et al.*, 2004). The high degree of regulation is due to the huge demand of water for irrigation purposes and human supply, which entails profoundly altering the natural flow regimes (Belmar-Díaz *et al.*, 2011) (see Chapter 2 for more information).

A total of 25 sampling sites in a stretch of river were located along upstream-downstream gradients, from the Upper Mundo River to the Mundo-Segura rivers confluence (52.2 km long) and from the Upper Segura River to Middle Segura (158 km) (Fig. 3.1). Each site was sampled by electrofishing three times in five years (2005-2010). The altitude range of the localities is 860-90 m.a.s.l., water conductivity varies between 364 and 1303 µS/s, and the average annual water temperatures ranges between 13.9°C in the upper stretches and 16.9°C in the lower stretches.

The fish assemblage in the Segura River basin is composed of both native and non-native species, but is dominated by the latter (Oliva-Paterna *et al.*, 2014). In the middle reaches where the *A. alburnus* inhabits, the ichthyophagous species northern pike *Esox lucius* L., 1758, pike-perch *Sander lucioperca* (L., 1758) and largemouth black bass *Micropterus salmoides* (Lacepèd, 1802) are also present. Along the river *A. alburnus* share resources and habitat with native cyprinids, such as southern Iberian barbel *Luciobarbus sclateri* (Günther, 1868) and southern Iberian chub *Squalius pyrenaicus* (Günther, 1868) and with several abundant non-native species, including pumpkinseed fish *Lepomis gibbosus* (L., 1758), Pyrenean gudgeon *Gobio lozanoi* Doadrio & Madeira, 2004, common carp *Cyprinus carpio* L., 1758, and Iberian straight-mouth nase *Pseudochondrostoma polylepis* (Steindachner, 1864).

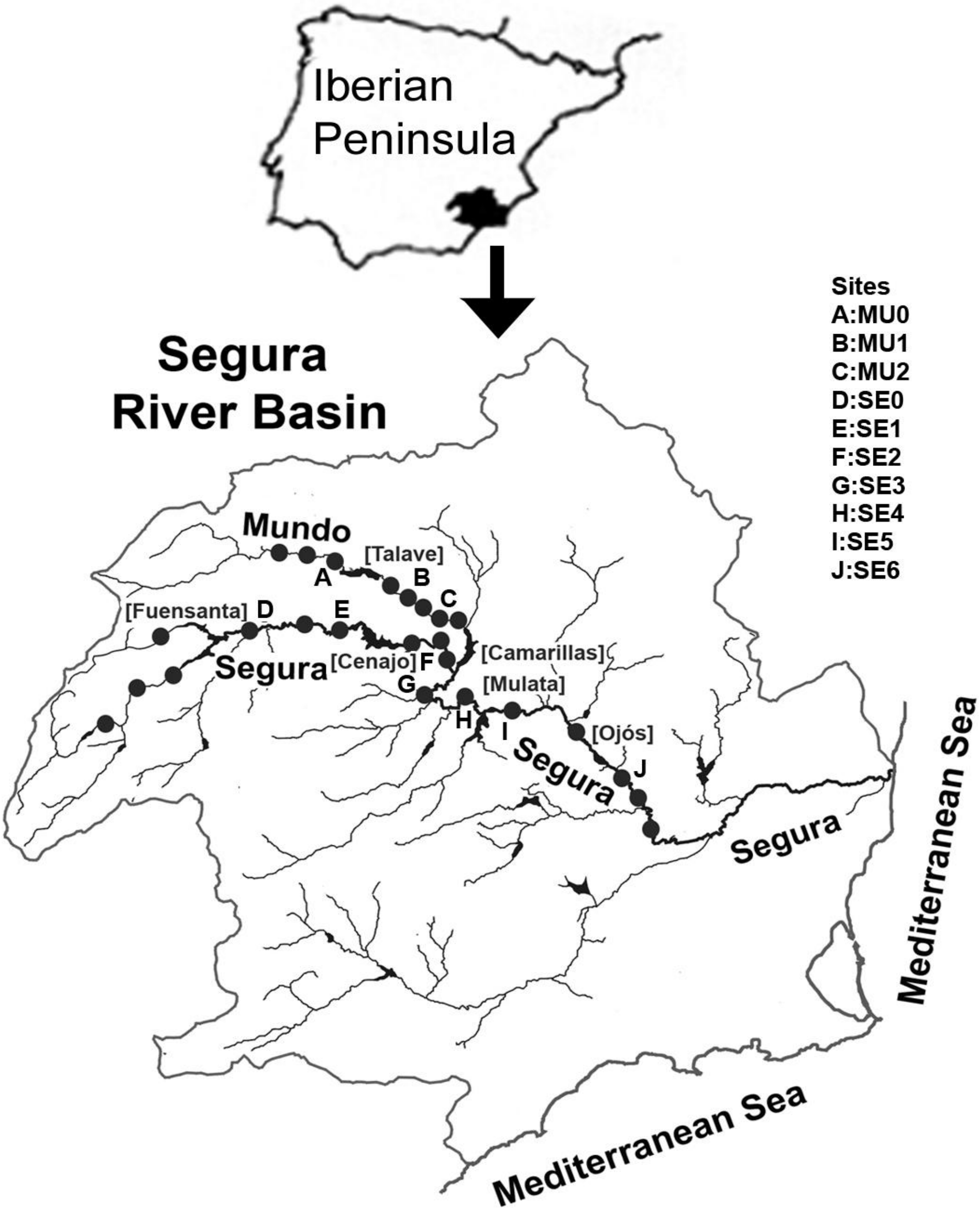

Figure 3.1. Location of the Segura River basin (SE Iberian Peninsula) and the 25 sampling sites (black points) where *Alburnus alburnus* occurrences were sampled (2005-2010). A-J: sampling sites located for the study of biological traits at population level (autumn 2010).

Field sampling and laboratory procedures

To study biological traits at population level, specimens from 10 sampling sites (Fig. 3.1 and Table 3.1) were captured by electrofishing following standard protocols (CEN, 2003) during autumn 2010 (mainly November). Sampling during this period avoided the capture of pre-spawning and spawning fish, and ensured that any variations in body condition were unaffected by gonad development. In order to obtain representative samples from each site in relation to the types of meso and microhabitats, the minimum wadeable sections covered at each sampling site were approximately 100 metres long. Fish collected at each sampling site were considered as independent populations for several reasons: the minimum distance along the river course between sampling sites exceeded 15 km and the main channels have several small weirs which restrict fish movements. However, the existence of a high population connection between individuals caught in the sampling sites of the same fluvial sector separated by large physical barriers (reservoirs), and a low connection between fish caught in adjacent fluvial sectors was assumed.

In accordance with Spanish regulations, total captures were sacrificed in an overdose solution of anaesthetic and stored on ice before preserved in 10% buffer formaldehyde solution for later processing in the laboratory. A total of 1285 *A. alburnus* were measured (Fork length; L_F ± 1 mm) and a sample of 474 individuals was processed to obtain the eviscerated mass (M_E ± 0.1 g) and organ masses (hepatic and gonad mass, M_H and M_G ± 0.001 g). Sex was determined by gonad examination, and age class using scales from the left side of the body of a total of 335 specimens. A Leica MZ 9.5 steromicroscope coupled with a digital camera (Leica DFZ 295) was used for counting annuli and LAS software version 3.5.0 to measure distances from focus to each annulus and scale radius length.

Populations were analysed at site and fluvial sector level along the longitudinal gradient from the upper part of the basin to the middle section of the Segura River in order to assess the intraespecific variability of the population traits. The fish parameters studied were: abundance (CPUE: Catch Per Unit Effort – number of fish caught per hour assuming a standardised sample area), size and age structure (by length-frequency distribution and back-length tables), growth rate (b-growth and growth index GI), somatic condition, hepatosomatic condition and reproductive investment.

Each sampling site was characterised by the following seven environmental variables: altitude (meters above sea level), ecological status *sensu* EU Water Framework Directive (Status) (with the following categories: 1 = high; 2 = good; 3 = moderate; 4 = poor), water conductivity ($\mu s\ cm^{-1}$), mean monthly temperature (ºC), mean daily flow (Mdf) ($m^3s\text{-}^1$) and flow variability (Fv) ($Q_{90\%}$-$Q_{10\%}$) (Table 3.1). The last three variables were calculated from a time series data of the 2009-2010 hydrological cycle obtained from the official monitoring service of the administrative organ of the Segura River basin (CHS, 2007).

Table 3.1. Habitat variability values of each sampling site where *Alburnus alburnus* populations were assessed. Altitude (m.a.s.l.); Ecological status (Status) (1-4); Water conductivity (Cond., ±0.1 µs cm^{-1}); Water temperature (Temp.,ºC); Mean daily flow (Mdf, m^3 s^{-1}) and Flow variability $Q_{90\%}$-$Q_{10\%}$ (Fv) from the cylce 2009-2010.

Sampling site	Code	Altitude (m.a.s.l.)	Status	Cond. (µs cm^{-1})	Temp. (ºC)	Mdf (m^3 s^{-1})	Fv
Cola Talave	MU0	540	2	619.3	13.87	5.11	-2.84
Puente Isso	MU1	460	3	674.3	14.10	13.84	-1.61
Blas García	MU2	430	3	687.7	14.10	13.84	-1.61
Bajo Fuensanta	SE0	470	1	340.0	14.67	10.20	-3.89
El Gallego	SE1	452	1	401.0	14.72	9.95	-1.57
Las Minas	SE2	306	2	579.3	14.28	5.18	-6.53
Cañaverosa	SE3	290	2	755.7	15.39	19.41	-1.16
El Esparragal	SE4	260	3	803.0	15.69	19.38	-1.15
Hoya García	SE5	200	2	860.7	16.43	19.07	-1.12
Azud de Ulea	SE6	112	4	1157.3	16.87	4.40	-1.59

Statistical analyses

The statistical analyses carried out to compare fish condition and reproductive investment included the application of an analysis of covariance (ANCOVA) using M_E, M_H and M_G as dependent variables, L_F as the covariate (log-transformation data) and maturity (individual with standard length ≥ 80 mm *sensu* Almeida *et al.* (2014)) and sex (male, female) as factors. Differences between dependent–covariate relationships were tested verifying that the covariate by-factor interaction (homogeneity of slopes) was significant ($P < 0.05$). If the covariate by-factor interaction was not significant, standard ANCOVA was applied to obtain predicted values. Variation in somatic condition, hepatosomatic condition and reproductive investment were only studied in mature fish (282 individuals) using the predicted values of M_E, M_H and M_G testing differences with an analysis of variance (ANOVA, Tukey's HSD post host tests) as proposed by García-Berthou & Moreno-Amich (1993).

Back-calculated lengths were obtained separately for each population according to the Fraser-Lee equation:

$$L_F n = [(Rn / R_T)\,(L_F - a)] + a$$

where L_F is at the capture time, $L_F n$ is L_F at age n, Rn is the scale radius at age n and a is the intercept of the linear relationship between the scale's total radius (R_T) and L_F. Differences between males and females in R_T-L_F regressions were tested by ANCOVA and a t-Student test was used to test differences between males and females for the back-calculated lengths.

Relative growth rates at each population was estimated by the mean values of growth index (GI) following the method used in previous studies that dealt with the same species (Masó *et al.*, 2016; Latorre *et al.*, 2018). For comparison of growth trajectories, the linearity in the relationships between ages and back-calculated L_F for each population was analysed at site level (b-growth). The homogeneity of the regression coefficients were tested (parallelism as the assumption of equal slopes) with an ANCOVA design that analysed the pooled covariate-by-factor interaction followed by an ANCOVA using sex and sampling sites as factors. Non-equal slopes indicate significant differences in the growth trajectories among populations.

The natural complexity of the habitat must be taken into account in studies of the sort presented, since many interacting variables may be involved simultaneously in the ecological variations along longitudinal gradients. This situation is best analysed through the use of multivariate statistics. In this study, a Principal Component Analysis (PCA) was applied with the varimax rotation method to the environmental variables matrix (ln or arcsin-transformed data) in order to extract independent components (henceforth PCs), which could be interpreted as gradients describing patterns in habitat variation along the longitudinal gradient of the rivers. To assess the usefulness of the PCA the Kaiser-Meyer-Olkin's measure (KMO) of sampling adequacy was used (values above 0.5). The scores of the selected habitat gradient (PCs) (Clavero *et al.*, 2009) were used to explore patterns of association (Regression models) between the habitat gradients and population traits.

Statistical analyses were performed with the SPSS® software package v. 15.0 and a significance level of 0.05 was accepted.

Results

Abundance and occurrence

Since 2010, *A. alburnus* has been found along a 35.4 km river stretch from the Upper Mundo River to the Mundo-Segura rivers confluence (67.8 % of the studied section) (Fig. 3.1 and 3.2). According to its frequency of occurrence in 2005 and 2010 throughout the sampled section from the Upper Segura River to Middle Segura River, the species showed a fast colonization rate, with a 24.8 % (39.2 km long) of the section occupied in 2005 and 84.4 % (133.4 km long) in 2010 (Fig. 3.1 and 3.2). Only in the fluvial sector above Fuensanta reservoir *A. alburnus* was not detected.

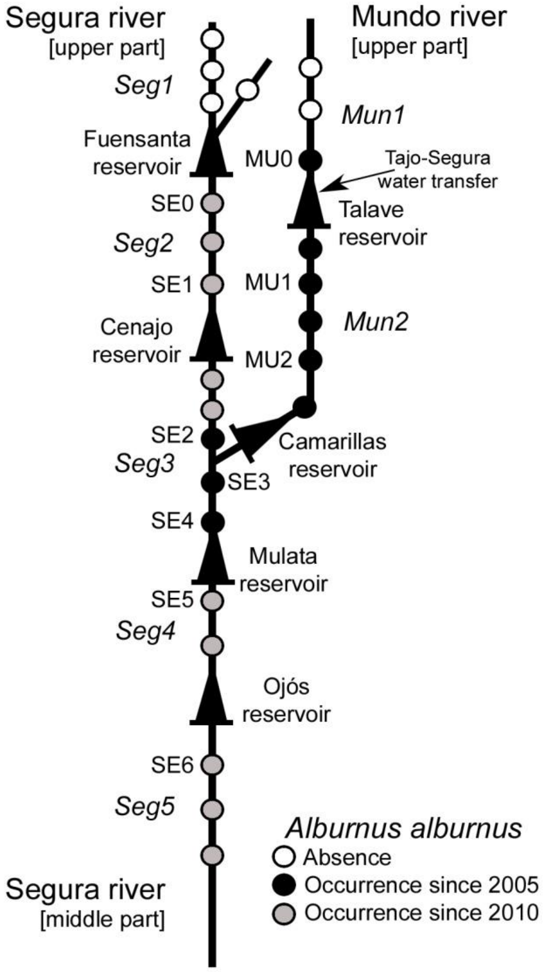

Figure 3.2. Diagram of the Segura and Mundo river axes and the 25 sampling sites (white, black and grey circles) where *Alburnus alburnus* occurrence was sampled (2005-2010). MU0 to MU2 and SE0 to SE6 were sampling sites selected for the study of biological traits at population level. The main physical barriers (principal dams) are shown (black triangles). White circles indicate sites where the species is absent, black and grey circles indicate the species presence since 2005 and 2010 respectively.

CPUE estimates for *A. alburnus* showed a high degree of spatial variation (Table 3.2), the highest relative abundances being found in the upper fluvial sectors above 400 m.a.s.l. (CPUEs > 100). Maximum values occurred in SE0 (320 CPUEs) and SE1 (226.5 CPUEs), located in the upper fluvial sector of the Segura River. Values were lower in sampling sites from the middle part of the basin with minimum values (SE2 and SE3) in the fluvial sector immediately below the largest reservoir of the basin (Cenajo reservoir) (Fig. 3.2 and Table 3.2).

Table 3.2. Population traits of *Alburnus alburnus* in sampling sites. Abundance (CPUE: Catch Per Unit Effort), mean and maximum fork length in cm (L_F), somatic condition, hepatosomatic condition and reproductive investment (estimated marginal means of M_E, M_H and M_G, log-transformed values) in immature (Immat.) and mature (Mat.) individuals.

Sampling site	Abundance (CPUE)	L_F		Estimated M_E		Estimated M_H		Estimated M_G	
		Mean	Max	Immat.	Mat.	Immat.	Mat.	female	male
MU0	198	12.5	16.7	--	2.79	--	-1.35	-1.16	-1.71
MU1	121.5	9.5	15.5	1.26	2.57	-2.80	-1.59	-2.51	-2.12
MU2	106	10.3	16.3	1.25	2.58	-2.67	-1.35	-1.43	-2.23
SE0	320	10.4	15.6	1.50	2.77	-2.87	-1.66	-1.16	-2.02
SE1	226.5	8.7	15.3	1.27	2.34	-3.32	-1.91	-1.36	-2.84
SE2	30.67	8.4	14.6	1.09	2.12	-3.20	-1.99	-2.86	-2.17
SE3	29.45	8.7	16.5	1.10	2.80	-2.73	-0.80	-0.38	-1.82
SE4	50	12.2	13.8	--	2.72	--	-1.52	-0.20	-1.80
SE5	71	8.1	12.6	0.93	2.18	-3.42	-2.34	-2.01	-2.75
SE6	55	7.5	11.0	0.77	2.02	-3.44	-2.18	-1.58	-3.06

Population traits

There were differences in size structure between sampling sites although a bimodal distribution pattern was evident along the longitudinal gradient (Fig. 3.3). MU0 and SE4 sampling sites located just above Talave and Mulata reservoirs, respectively (Fig. 3.1 and Fig. 3.2), were the only sites without individuals measuring less than 8 cm L_F, but clear presence of young of the year (0+) were not observed at any sampling site.

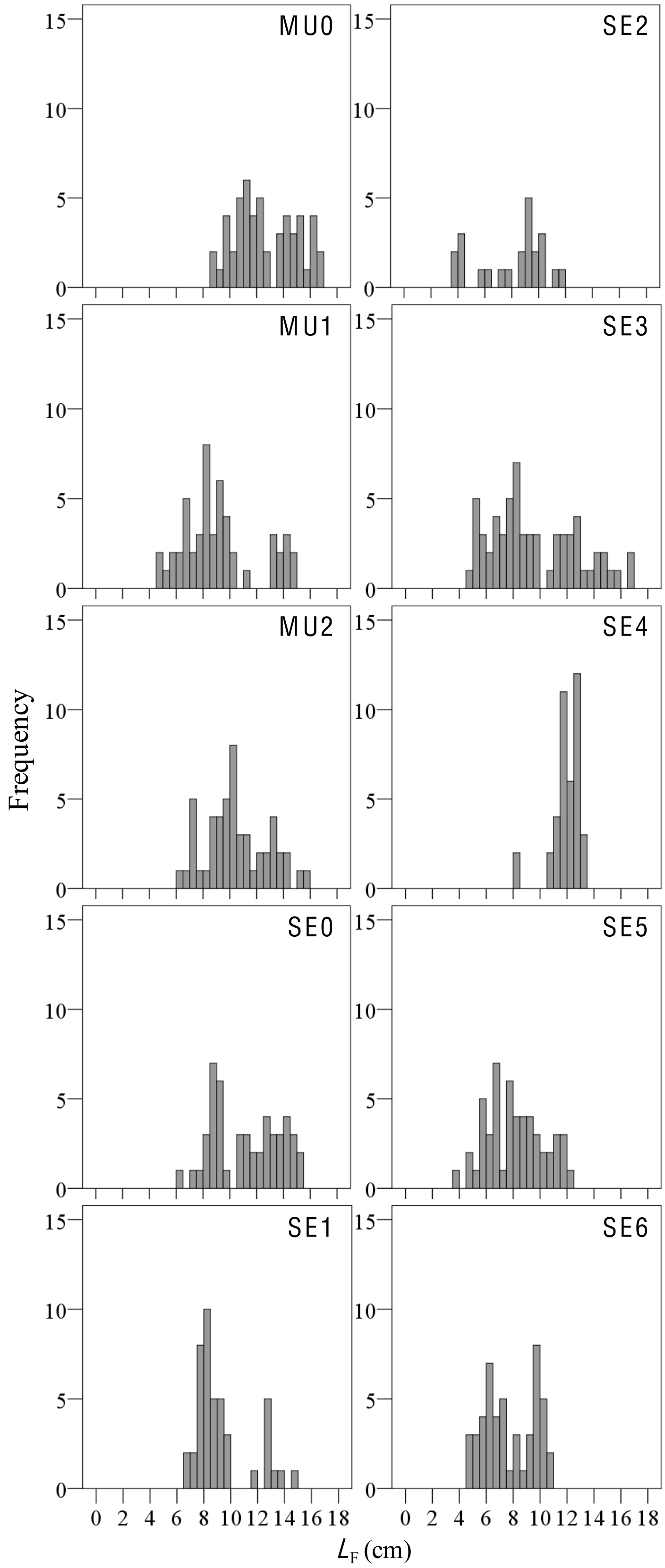

Figure 3.3. Length-frequency distributions (L_F; 1 cm length groups) of *Alburnus alburnus* in the sampling sites (MU0 to MU2 and SE0 to SE6) selected for the study of biological traits at population level.

When age was determined by scales, five age groups (0+ to 4+) were identified for both sexes, although only one male (caught in MU1) presented an age of 4+. Populations (site level) showed older females (4+) only in sampling sites where the species had inhabited since 2005 (Fig. 3.2), while in the more recently invaded areas the oldest captures were individuals from the 3+ age group (Table 3.3 and Fig. 3.2). *Alburnus alburnus* individuals ranged from 3.5 cm to a maximum L_F of 16.9 cm (a female caught in MU0), although males (L_F 10.9 ± 0.3 cm) were significantly longer than females (L_F 10.1 ± 0.4 cm) (ANOVA, $F_{(1,\ 397)} = 9.15$, $P = 0.003$). Significant spatial effects (at site level) were detected (ANCOVA, $F_{(1,\ 9,\ 397)} = 2.97$, $P = 0.002$; sex x site), with a decrease in the average L_F in populations along the longitudinal gradient; larger sizes (> 13 cm L_F) were not captured at lower sampling sites (Fig. 3.3 and 3.4).

Back-calculated lengths, the slopes of the back-calculated L_F - age relationships and growth index at sampling site level are presented in Table 3.3. Sex had no significant effect on back-calculated lengths (ANOVA, $F_{(1,\ 578)} = 2.27$, $P = 0.132$) or on the relationships (ANCOVA, $F_{(1,\ 9,\ 578)} = 0.30$, $P = 0.974$; sex x site). There were significant differences among sampling sites on slopes (b-growth parameter) of the relationships (ANCOVA, $F_{(1,9,\ 638)} = 3.65$, $P < 0.001$ in slope). *Alburnus alburnus* growth was fastest in populations of the upstream sector of the Segura (SE0 and SE1) (Table 3.3).

Table 3.3. Population traits of *Alburnus alburnus* at sampling sites. Mean back-calculated fork length (cm) at ages L_F (n), slopes of L_F -age relationships (b-growth), growth index (GI, % ± IC 95%) and the sex-ratio (males: females). (*: Significant differences from 1:1. $P < 0.05$).

Sampling site	L_F (1+)	L_F (2+)	L_F (3+)	L_F (4+)	b-growth	GI	Sex-ratio
MU0	5.8	8.2	10.7	13.5	24.6	116.0 ± 15.5	0.85
MU1	5.5	7.8	10.8	12.9	25.6	114.5 ± 9.5	0.57*
MU2	5.6	8.0	10.7	13.5	25.3	114.1 ± 15.7	0.59*
SE0	6.0	8.6	12.1	-	28.8	122.4 ± 7.9	0.67
SE1	6.1	8.4	11.4	-	25.7	120.7 ± 11.9	0.48*
SE2	5.3	7.4	9.0	-	19.1	104.3 ± 15.7	1.29
SE3	5.6	8.1	10.8	11.4	24.2	116.8 ± 11.1	0.86
SE4	5.9	8.4	10.3	-	23.1	116.3 ± 18.0	1.44
SE5	5.4	8.0	9.5	-	23.5	109.1 ± 8.7	0.67
SE6	5.7	7.7	9.5	-	19.5	111.1 ± 4.0	0.78

The overall sex-ratio (171 males, 227 females) was significantly skewed towards females (χ^2= 7.88, P = 0.005), although only in three sampling sites from the upper sectors (MU1, MU2 and SE1 sampling sites) females were significantly dominant (Table 3.3).

Maturity had a significant effect on the organosomatic indexes and sex only significantly affected the reproductive investment values (ANCOVA, P < 0.05; Table 3.4). Indeed, the ANCOVA analysis (initial or final design in Table 3.4) pointed to significant differences in estimated marginal values of M_E, M_H and M_G (Table 3.4). Patterns of variation in relation to the environmental gradients are shown in figures 3.4 and 3.5.

Table 3.4. Results of ANCOVA analyses of population traits of *Alburnus alburnus*. Fork length is the covariate, and maturity, sex and sampling site are the factors. Degrees of freedom, *F*-statistics and *P* values (significant effects in bold) are presented.

	Dependent variables											
	Eviscerated mass			Hepatic mass			Gonadal mass					
Source	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*			
	All individuals											
Length	1	14444.9	**<0.001**	1	419.17	**<0.001**	1	432.39	**<0.001**			
Maturity	1	33.97	**<0.001**	1	8.27	**0.004**	1	8.23	**0.004**			
Length x maturity	1	36.15	**<0.001**	1	10.16	**0.002**	1	10.02	**0.002**			
	Mature individuals											
Length	1	11411.6	**<0.001**	1	501.93	**<0.001**	1	854.73	**<0.001**			
Sex	1	0.74	0.389	1	0.01	0.908	1	5.91	**0.016**			
Length x sex	1	1.25	0.265	1	0.001	0.990	1	10.35	**0.001**			
	Immature individuals											
Length	1	1025.44	**<0.001**	1	15.13	**<0.001**	1	66.25	**<0.001**			
Sex	1	0.12	0.723	1	0.002	0.96	1	13.18	**<0.001**			
Length x sex	1	0.16	0.689	1	0.001	0.97	1	11.08	**<0.001**			
							Males			Females		
	Mature individuals						df	*F*	*P*	df	*F*	*P*
Length	1	4054.10	**<0.001**	1	230.90	**<0.001**	1	111.3	**<0.001**	1	15.03	**<0.001**
Sampling site	9	1.58	0.122	9	2.54	**0.008**	9	1.40	0.194	9	2.93	**0.004**
Length x Sampling site	9	1.33	0.224	9	2.14	**0.027**	9	1.56	0.134	9	3.03	**0.003**
Final design (no interaction)												
Length	1	12241.7	**<0.001**				1	344.9	**<0.001**			
Sampling site	9	15.62	**<0.001**				9	3.77	**<0.001**			
	Immature individuals											
Length	1	1356.9	**<0.001**	1	9.55	**0.003**						
Sampling site	7	4.07	**0.001**	7	2.33	**0.030**						
Length x Sampling site	7	4.25	**<0.001**	7	2.57	**0.018**						

Relationship between population traits and environmental variables

Information on habitat characteristics was summarised in a PCA (KMO = 0.60), which produced two gradients (PC1 and PC2) with eigenvalues > 1 that explained 82.3% of the original variation within the data set at site level (Table 3.1). PC1 explained 57.4% of the variation and described a gradient running from the upper sampling sites (higher altitude) (Correlation coefficient r = -0.86; $P = 0.001$) to sites of poorer ecological status (r = 0.84; $P = 0.002$), higher water conductivity values (r = 0.98; $P < 0.001$) and higher monthly temperatures (r = 0.90; $P < 0.001$), in short, a habitat quality deterioration trend correlated to the longitudinal gradient in the basin. PC2, which was positively associated with mean daily flows (r = 0.89; $P = 0.001$) and flow variability (r = 0.88; $P = 0.001$), explained 24.9% of the variation.

Alburnus alburnus abundance presented significant relationships with both habitat gradients (PC1 and PC2) (Fig. 3.4). CPUEs at site level showed a significant decreasing relationship with positive values in PC1, but a quadratic relation with the flow gradient (PC2), abundance peaking at intermediate values of the gradient. PC1 gradient had a quadratic negative (and linear) effect on maximum captured sizes at each site (L_F max), but no effects on mean L_F (Fig. 3.4). The parameter (b-growth) was negatively related (linear effect) to the longitudinal gradient (PC1) and showed a quadratic relationship with flow gradient (PC2), presenting higher values in the middle part of the gradient (Fig. 3.4). GI showed a similar pattern although it did not present significant levels.

All the organosomatic indexes depended significantly on L_F (Table 3.4) and, after accounting for this, the somatic condition, hepatiosomatic condition and reproductive investment significantly differed among populations (sampling sites). However, only for the somatic condition in juveniles (estimated M_E values) showed a linear regression with PC1 significant (Fig. 3.5).

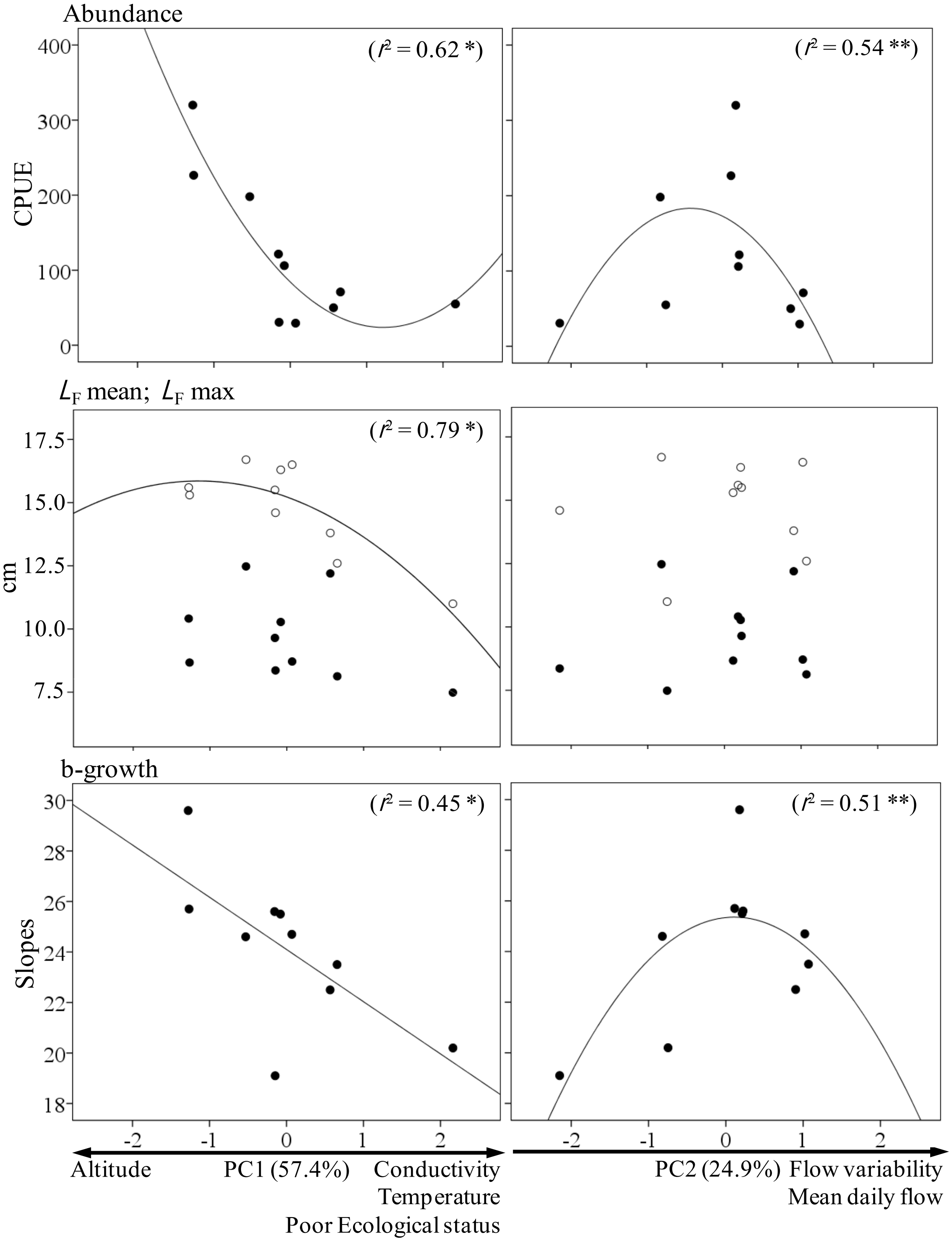

Figure 3.4. Relationships between *Alburnus alburnus* abundance, mean fork length (black circles), maximum fork length (white circles) and b-growth at population level and the habitat gradients (PC1 and PC2). Explained variance of each PC is presented and the interpretation of PCs is based on the significantly correlated original variables. Significant relationships (linear or quadratic) are marked with continuous lines (*: significance level $P < 0.05$; **: marginal significance level $P < 0.1$).

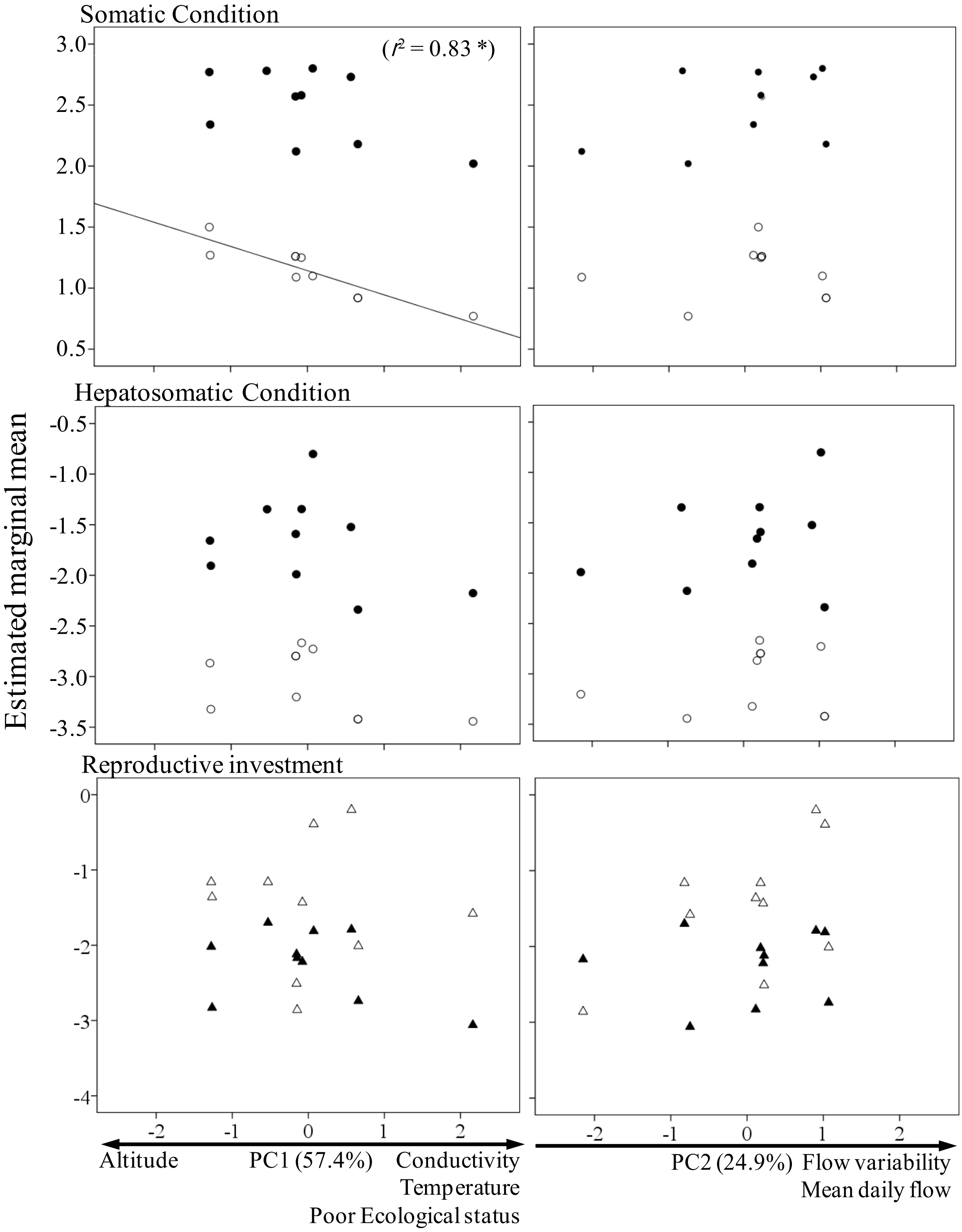

Figure 3.5. Relationships between *Alburnus alburnus* somatic condition, hepatosomatic condition, and reproductive investment (estimated marginal means of M_E, M_H and M_G, respectively) at population level and the habitat gradients (PC1 and PC2). Explained variance of each PC is presented and the interpretation of PCs is based on the significantly correlated original variables. Mature and immature individuals are marked with black and white circles, respectively. Black and white triangles indicated male and female values respectively. Significant relationships (linear or quadratic) are marked with continuous lines (*: significance level $P < 0.05$; **: marginal significance level $P < 0.1$).

Discussion

The rapid expansion of *A. alburnus* in the Segura River basin is another evidence of its invasiveness potential related to colonization velocity. Despite being typical of the open waters of lakes and medium-large rivers in its native range, the previously studied Iberian populations of *A. alburnus* displayed its ability to inhabit small streams (Almeida *et al.*, 2014, 2017; Masó *et al.*, 2016). In the present study, the establishment of *A. alburnus* populations along more than 168.8 km (133.4 km of the Segura River, 35.4 km of the Mundo River) in one of the most regulated Iberian river basins and inhabiting lotic environments (i.e. fluvial stretches) has been confirmed. In fact, the results show that *A. alburnus* can reach high abundances in different habitat conditions although they were significantly related to habitat quality deterioration and flow gradients. Thus, in agreement with Almeida *et al.* (2014), although *A. alburnus* is considered a limnophile suprapelagic species, the occurrence and abundance data obtained for the Segura River basin showed that the species also develops well in lotic environments, confirming its wide phenotypic plasticity as regards of habitat requirements. This is a common population trait of invasive fishes, which certainly facilitates their invasion process (Sakai *et al.*, 2001).

Reservoirs have been used for introducing exotic fish species (Elvira & Almodóvar, 2001; Clavero *et al.*, 2013). In the case of the Iberian populations of *A. alburnus*, a positive correlation was established between their dispersion and regulated rivers because large reservoirs could be acting as source populations to colonize river stretches (Vinyoles *et al.*, 2007). In the Segura River basin, its colonization has been attributed to fish dispersion along an interbasin system that flows into the Talave reservoir (Fig. 3.2) or to its deliberate or accidental introduction by anglers since sport fishing is popular in reservoirs from the upper part of the basin (Andreu-Soler *et al.*, 2004). The results suggest that the presence of *A. alburnus* in some of these fluvial sectors is related to deliberate introductions. Moreover, higher abundances of *A. alburnus* were detected in fluvial sectors of the upper part of the basin located between the largest reservoirs where natural flow regimes are highly affected (Belmar-Díaz *et al.*, 2011). Longitudinal connectivity is also highly affected by the presence of small longitudinal barriers (i.e. weirs) which implies an increase in lentic and homogeneous environments favouring the expansion of introduced limnophilic species (Clavero *et al.*, 2013). In the Segura River basin, lentic stretches created by weirs also probably act as shelter habitats for *A. alburnus* populations because a negative relationship between *A. alburnus* abundance and downstream distance to the closest lentic habitat (weir or reservoir) was obtained (Linear regression, b-slope = -0.35, $r^2 = 0.43$, $P = 0.04$).

Size population structure showed two mature cohorts along the whole longitudinal gradient. This bimodal pattern has been found in previous studies on the species in both non-native regions (population in rivers and reservoirs in the Iberian Peninsula) (Almeida *et al.*, 2014) and native regions (lake populations in Hungary) (Bíró & Muskó, 1995). In a similar way, the age structure of *A. alburnus* specimens caught in the Segura

River basin (from 0+ to 4+ age groups) does not differ from that observed in other Iberian studies (Almeida *et al*., 2014, Latorre *et al*., 2016), but showed a significant contrast with some native areas. As an example, the native populations which show up to ten age classes (9+) in Bíró & Muskó (1995). Moreover, higher longevity and larger size cohorts were more common in fluvial sectors where the fish had resided longer (Mundo River). These results could be related to earlier stages of invasiveness of the species in fluvial sectors where the residence time is shorter. A higher reproductive effort in newly invaded sectors where the species is not fully adapted probably triggered higher mortality of larger individuals when environmental conditions worsened after the summer (Hutchings *et al*., 1999; Bøhn *et al*., 2004). A similar result in the reproductive investment indicator was not obtained probably because autumn data outside the reproductive period of the species are not the most suitable for such assessment.

The population dynamics in the early stage of invasiveness present different life strategies compared to well-established populations (Ribeiro *et al*., 2008). For instance, Bøhn *et al.* (2004) showed higher growth rates in invasive fish during the initial invasion process compared with sites where there were established populations. It is expected that in the early stages of the establishment process, with low densities and slight intraspecific competition, *A. alburnus* presents high growth rates and a high reproductive investment. Accordingly, in the present study two sampling sites (SE0 and SE1), associated with the group with shorter residence time, and located in the upper part of the basin showed the highest mean back-calculated lengths at age and, also, the fastest growth (higher b-growth and GI values). However, another two sites where the species also showed short residence times (SE5 and SE6), but located in the lowest part of the longitudinal gradient and so in fluvial sectors with a poorer ecological status (PC1 gradient), the species showed lower back-calculated lengths and also lower growth rates. In general terms, GIs estimated in this study were higher than values obtained in Latorre *et al.* (2018) for the same basin and other rivers of the Iberian Peninsula (Masó *et al*., 2016).

According to some postulates in ecology of freshwater fish (Wootton, 1998), the growth traits pattern of *A. alburnus* populations would be expected to reflect differences in habitat condition along the longitudinal gradient. In fact, decreasing altitude and flow constraints combined with increasing temperature, and the presence of deep-water areas, as a sort of hydraulic refuges (Bouchard *et al*., 1998), and with increased nutrient availability downstream were expected to positively influence the growth rates of *A. alburnus*, observed in others Cyprinids (Tedesco *et al*., 2009). Conversely, abundance, maximum length and growth in *A. alburnus* populations showed a negative significant relationship with the longitudinal gradient (PC1; Fig. 3.4) running from the sites located in the upper part of the Segura River basin to sites in the middle part with higher water temperatures, higher water conductivity values and poorer ecological status. These findings could be related to the residence time of the studied populations but also, probably, with the increase in environmental stress along the longitudinal gradient. In fact, agrochemical and nutrient inputs (via run-off) are high in the fluvial sector occupied by *A. alburnus* because the middle part of the Segura River basin is one of the most important irrigated agricultural areas of the Iberian Peninsula (Grindlay *et al.*, 2011). This lead to a decrease in ecological status and an increase in water conductivity values along the longitudinal gradient, which could have negative effects on *A. alburnus* densities and growth despite its generalist character (Horppila & Kairesalo, 1992).

Despite *A. alburnus* being considered as a limnophile suprapelagic fish (Harby *et al.*, 2007), in other studies in Mediterranean streams the species has been categorized as eurytopic and preferably dwelling in run-type habitats with an appreciable flow velocity (Muñoz-Mas *et al.*, 2016) which would also explain better the rapid spread of *A. alburnus* in the Iberian systems (Vinyoles *et al.*, 2007). Certainly, flow regime is one of the fundamental attributes of lotic ecosystems, and has profound effects on physical, chemical and biological characteristics, which promotes significant changes among population traits (Wootton, 1998). In *A. alburnus* populations of the Segura River basin, abundance and the growth parameters were significantly dependent on the ecological variables related to water flow. Sampling sites with medium values of flow variability and mean daily flow provided the highest fish density and growth rates. This flow effect could also partially explain the lack of homogeneity observed in growth among populations with similar residence times, as previously mentioned. Nevertheless, the results presented here are a first approach to assess the life-history variability of *A. alburnus* populations under different flow regulation conditions, and further research should include additional approaches to relate such variability with regulation types or with seasonal variation effects. Piscivorous species may exert a strong predatory pressure on *A. alburnus* populations (Almeida & Grossman, 2014) and, thus, acting as a leading factor influencing in traits. Similar predatory pressure among the studied populations was assumed because of the similarity in the fish community composition (Oliva-Paterna *et al.*, 2014). However, community effects should be more closely investigated.

Almeida *et al.* (2014) found a seasonal effect on the sex-ratio, with females being predominant in lotic habitats during autumn. The present results are partially similar to that work because the sex-ratio in the Segura River basin as a whole was significantly skewed towards females, although seven of the studied populations showed an equal sex-ratio. Proportion of sexes is strongly influenced by environmental factors and seasonality, as was observed in the introduced *A. alburnus* populations (Fouzia & Abdeslem, 2012). In the Iberian Peninsula, other studies on the target species have frequently pointed to a predominance of males, although many of them were made with spring data (Masó *et al.*, 2016; Latorre *et al.*, 2018), which are not comparable with those of the present study.

The somatic and hepatosomatic condition, and also the reproductive investment, differed substantially between the populations studied. Differences in the estimated values of M_E, M_H and M_G were probably caused by differences in habitat conditions, but little or nothing correlated with the longitudinal gradient (only a significant relationship between juvenile fish condition and the PC1 gradient). The short sampling period in November prevented the capture of prespawning and spawning fish and thus ensured that the variability of the estimated values were not related to seasonal variations in the conditions of *A. alburnus* (Almeida *et al*., 2014). However, it also means that the estimated data of M_G is not suitable for evaluating reproductive investment. Changes in the ecological status, water conductivity and temperature along the longitudinal gradient probably affect *A. alburnus* condition, as has been observed in other fish species (De Miguel *et al*., 2013). Moreover, other studies in the same river basin showed that the ecological features that accounted for most of the variation in fish condition were variables related to flow and water conductivity (Oliva-Paterna *et al*., 2003). The results concerning the somatic and hepatosomatic condition of *A. alburnus* also showed no relationships with flow characteristics and their variability suggested high phenotypic plasticity at population level, which has previously been proposed (Alemida *et al*., 2014; Masó *et al*., 2016; Latorre *et al*., 2018). Probably, changes in habitat characteristics at mesohabitat scales which are not included in the present study led to the variability of the *A. alburnus* conditions.

In agreement to Tedesco *et al.* (2009), the results of the present study have clear consequences for interpretation in large-scale comparative studies, particularly those focusing on latitudinal variability in invasive freshwater fishes. In general, studies comparing invasive fish populations from different basins, including the only study of this type with *A. alburnus* (Latorre *et al*., 2018), often use data obtained from the literature or from sampling designs without accounting for position in the longitudinal river gradient (i.e. Cucherousset *et al*., 2008).

The rapid spread and establishment of *A. alburnus* in highly regulated Mediterranean basins can be attributed, in part, to their adaptive capacity in changing population traits in response to environmental condition but also to the lentic shelter habitats created by the impact of flow regulation (i.e. weirs), although this subject should be more closely investigated. Non-native fish species with lower trophic positions are more efficient in obtaining energy from basal trophic groups (Gido & Franssen, 2007); moreover, *A. alburnus* shows a wide trophic plasticity (Almeida *et al*., 2014, 2017), which concurs with the higher success of the species in the Segura River basin.

Conclusions

Alburnus alburnus is widely established in the Segura River basin and this study confirms a high occurrence of the species in river stretches which are suboptimal habitats according to its ecological requirements. Moreover, flow regulation and the presence of impoundment structures seem to be affecting abundances of *A. alburnus.*

Size population structure showed a bimodal pattern also found in previous studies on the species in both non-native and native regions. The age structure (from 0+ to 4+ age groups) turned out similar to other Iberian locations, but much smaller than in its native areas. Longer residence time determined populations with larger size cohorts and older fish. The absence of larger ages in particular areas concur with earlier stages of invasiveness process, in which larger individuals, probably after a high reproductive effort, could be suffer higher mortality.

Abundance, maximum length and growth in *A. alburnus* populations showed a negative significant relationship with the longitudinal gradient. These parameters showed lower values in areas where environmental stress was higher, with warmer water temperatures, higher water conductivity and poorer ecological status. Flow regulation conditions had effect on abundance and growth parameters, providing more abundances and faster growth in areas with medium flow variability and mean daily flow. This result suggests that flow variability is an important factor conditioning population traits development.

In summary, the main results support the hypothesis that the wide phenotypic variability in population traits previously described for *A. alburnus* enables it to adapt and to establish new populations in highly regulated Mediterranean-type rivers. This phenotypic plasticity seems to be an important factor in its invasion processes. According to Bøhn *et al.* (2004), the success of an invasive species is probably more a consequence of variability in their population traits than of genetically determined life features.

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Chapter 4

Assessing effects of flow regulation on size-related variables of freshwater fish in a Mediterranean basin

Introduction and objectives

In rivers, natural hydrological regime plays an important role on the environmental aquatic structure (Poff, 1997; Lytle & Poff, 2004). River discharge can act as an ecological filter determining the evolution and distribution of organisms inhabiting particular areas (Poff, 1997; Weiher *et al.*, 2011; Gutiérrez-Cánovas *et al.*, 2015). Thus, magnitude and variability of flow regime results a strong driving force on the development of fish fauna (Lytle & Poff, 2004; Mims & Olden, 2013) and their effect on fish has been widely recorded (Langerhans & Reznik, 2009; Haas *et al.*, 2010; Mims & Olden 2012; Niu *et al.*, 2012; Bennet & Kozak, 2016).

Historically, natural hydrological regimens have been intensely modified. Building of dams to supply a wide range of human needs has contributed to, currently, the impoundment of a large number of major river systems (Vörömarty *et al.*, 2010; Lehner *et al.*, 2011). In Mediterranean basins, flow regulation is stronger due to the seasonal climatic patterns displayed in these areas with floods in autumn and spring and dry periods in summer, which have also encouraged the damming for floods control. The presence of dams alters flow regime patterns and diminishes natural flow disturbances, reducing or losing flow seasonality and variability downstream of dams (Poff *et al.*, 2007). Moreover, artificial structures modify the river morphology, generating new artificial-flow habitats with suboptimal conditions, which could create new niches or refuge areas for more generalist and opportunistic faunas (Bunn & Arthington, 2002; Olden *et al.*, 2006; Mims & Olden, 2013). Other impacts provided by longitudinal fragmentation of rivers due to the presence of artificial barriers are changes in water quality and temperature, alteration of physical surrounding habitats and food availability and, finally, modification of the natural dynamic and functionality of rivers (Richter *et al.*, 1996; Bunn & Arthington, 2002; Vörömarty *et al.*, 2003).

Freshwater fish have had to adapt to this new framework of environmental conditions in aquatic systems promoted by river regulation. Previous studies have analysed the effect of flow on freshwater fish at different organization levels, such as individual level (Langerhans *et al.*, 2003; Franssen, 2011), population level (Tedesco *et al.*, 2008; Mims & Olden, 2013; Vasconcelos *et al.*, 2014) or at fish assemblage level (Alexandre & Almeida, 2010; Franssen & Tobler, 2013). Moreover, the presence of dam is acting as a filter for some life strategies favouring some life strategies (equilibrium strategists) against others (opportunists) (Olden *et al.*, 2006; Carlisle *et al.*, 2011; Mims & Olden, 2013). Even small obstacles could cause changes in the composition and structure of fish assemblage (Alexandre & Almeida, 2010; Benejam *et al.*, 2014). These changes on fish community structure have often implied a reduction of native species and an increment of non-native species, whose generalist life histories favour them to colonize altered areas (Bunn & Arthington, 2002; Olden *et al.*, 2006; Mims & Olden, 2013).

Population size structure can be modulated by both biotic and abiotic factors (De Leeuw *et al.*, 2003; Brucet *et al.*, 2010). Thus, any ecological factor affecting the growth, mortality and trophic interactions of organisms would influence on body sizes and ultimately on population size structure (De Roos *et al.*, 2003; Savage *et al.*, 2004; Brown *et al.*, 2007). Size structure of fish assemblages and populations are recognised as important ecosystem health indicators (Petchey & Belgrano, 2010; Emmrich *et al.*, 2014) and may be used to evaluated the effect of anthropogenic perturbation on fish (Aparicio *et al.*, 2011; Emmrich *et al.*, 2011; Brucet *et al.*, 2013; Murphy *et al.*, 2013; Benejam *et al.*, 2014). However, along the natural environmental gradient of rivers (i.e. longitudinal, altitudinal) many ecological processes can vary (Carmona-Catot *et al.*, 2011) and it could also reflect changes on size structures (Fitz *et al.*, 2016; Benejam *et al.*, 2018). Size-related variables as mean and maximum length, size diversity, variance, skewness or slope of size spectrum have been already used as useful variables to assess size structure of fish populations and assemblages (Murphy *et al.*, 2013; Emmrich *et al.*, 2014; Arranz *et al.*, 2015; Benejam *et al.*, 2015). Studies on size structure have been mainly focused on big scale environmental gradients (Emmrich *et al.*, 2011, 2014; Arranz *et al.*, 2015), on different types of anthropogenic perturbations as water quality, land use or population density (Brucet *et al.*, 2013; Murphy *et al.*, 2013; Benejam *et al.*, 2015) and on size structure of fish assemblages (Emmrich *et al.*, 2011, 2014; Brucet *et al.*, 2013; Benejam *et al.*, 2015, 2018). Flow alteration is another factor that could modify size-related variables of fish populations or assemblages; in fact, lower sizes in fish have been associated with water withdrawal (Xu *et al.*, 2010; Uzunova *et al.*, 2012; Benejam *et al.*, 2014). However, despite population size structures have proved to reflect changes of environmental factors, studies concerning the effect of flow regulation on size-related variables of river specialist fish are scarce (Murchie *et al.*, 2008; Xu *et al.*, 2010; Benejam *et al.*, 2014; Merciai *et al.*, 2017).

Fish species present in rivers have been developed under certain environmental conditions (Lytle & Poff, 2004; Mims & Olden, 2013). Historically, researchers have classified into common groups species behaving in a similar manner or sharing ecological requirements as habitat (Kryzhanovsky, 1948), trophic resources (Karr *et al.*, 1986), reproduction (Balon, 1975) or environmental guilds (Welcomme *et al.*, 2006). In this study, chosen target species may be consider as included in different ecological typologies of freshwater fish which correspond to different environmental guilds, mainly focused on flow and habitat requirements (Welcomme *et al.*, 2006). These target species were: *Lepomis gibbossus* (L., 1758) as limnophilc species, typical of lentic environments and pool areas with slow flow (Scott & Crossman, 1973; Copp & Fox, 2007); *Alburnus alburnus* (L., 1758) as limnophile suprapelagic species, which inhabits large rivers and big lakes where occupies the top area of water column (Haberlehner, 1988; Freyhof & Kottelat, 2008), and the rheophilic species *Luciobarbus sclateri* (Günther, 1868) and *Gobio lozanoi* Doadrio & Madeira, 2004, which prefer areas with moderate-high flows and present benthic habits. *Luciobarbus sclateri* was selected as the native species of the southern Iberian basins and it is typical of medium areas of rivers (Torralva *et al.*, 1997; Encina *et al.*, 2006), while *G. lozanoi* is an allochthonous species (Doadrio *et al.*, 2011).

The main goal of this study was to assess the effect of flow regulation on population size structure of different ecological types of freshwater fish along the longitudinal gradient in a highly regulated Mediterranean river basin. This assessment was carried out through the following objectives:

a) To analyse size-related variables of four freshwater fish (1 limnophilic, 1 suprapelagic, and 2 rheophilic) widely distributed along the longitudinal gradient of the Segura river basin.
b) To assess the effects of flow regime variability and the presence of artificial-flow habitats on size-related variables.

Material and methods

Study Area

The Segura River basin (18870 km^2 drainage area) is located in the south-east of Spain (Fig. 4.1) and represents one of the most arid zones of the Iberian Peninsula and, probably, of Europe within the Mediterranean area (Geiger, 1973). The basin is characterised by Mediterranean climate, displaying an elevated seasonal and annual flow variation, with flash floods in autumn and spring and severe droughts in summer (CHS, 2017).

Flood control and water demand have been the main reasons promoting dams building in this region. Currently, Segura River basin is one of the most regulated basins in Europe (MMA, 2004) showing 33 large dams (more than 15 m height) (CHS, 2017) and 121 weirs higher than 2 m. Water demands from agricultural, urban, industrial use and environmental requirements exceed a 224% of available water in the basin (Zimmer, 2010). Under these circumstances, the natural flow regime is highly altered, with a decrement of flow magnitudes and a reverse seasonal flow pattern in some areas (Belmar-Díaz *et al.*, 2011) (see Chapter 2 for more information).

The study area is located in the upper and middle part of the basin. The 29 sampling sites were distributed in 8 hydrological sectors (HS) with different flow regulation patterns along the longitudinal gradient of the Segura River basin, in the main rivers Segura and Mundo and in two small headwater tributaries, Taibilla and Tus rivers (Fig. 4.1). Sampling sites sharing the same flow conditions derivate from the presence or absence of anthropogenic structures were classified in three types of stretches (TS) and categorised as: (1) free-flow habitats (natural mesohabitats) (no artificial structures are present), (2) sites with bypass artificial-flow habitats related to weir points, and (3) sites with lentic artificial-flow habitats next to the tail of reservoirs or just downstream of the dams (Table 4.1 and Fig. 4.1).

Fish assemblage has suffered a great change in the last 25 years (Oliva-Paterna *et al.*, 2014). Currently, introduced species are abundant and widely distributed in the basin (Andreu-Soler *et al.*, 2006). The native southern Iberian barbel, *L. sclateri*, is the most common species in whole study area (frequency of occurrence of 95.5% in the upper part and 86.7% in the middle part). Followed by the non-native species Pyrenean gudgeon, *G. lozanoi*, with a 93.3%, bleak, *A. alburnus*, with 86.7% and, finally, pumpkinseed fish, *L. gibbosus*, with 80% of occurrence in the middle part of the basin (Oliva-Paterna *et al.*, 2014). Due to their high occurrence and wide distribution in the study area, these four species have been selected for this study.

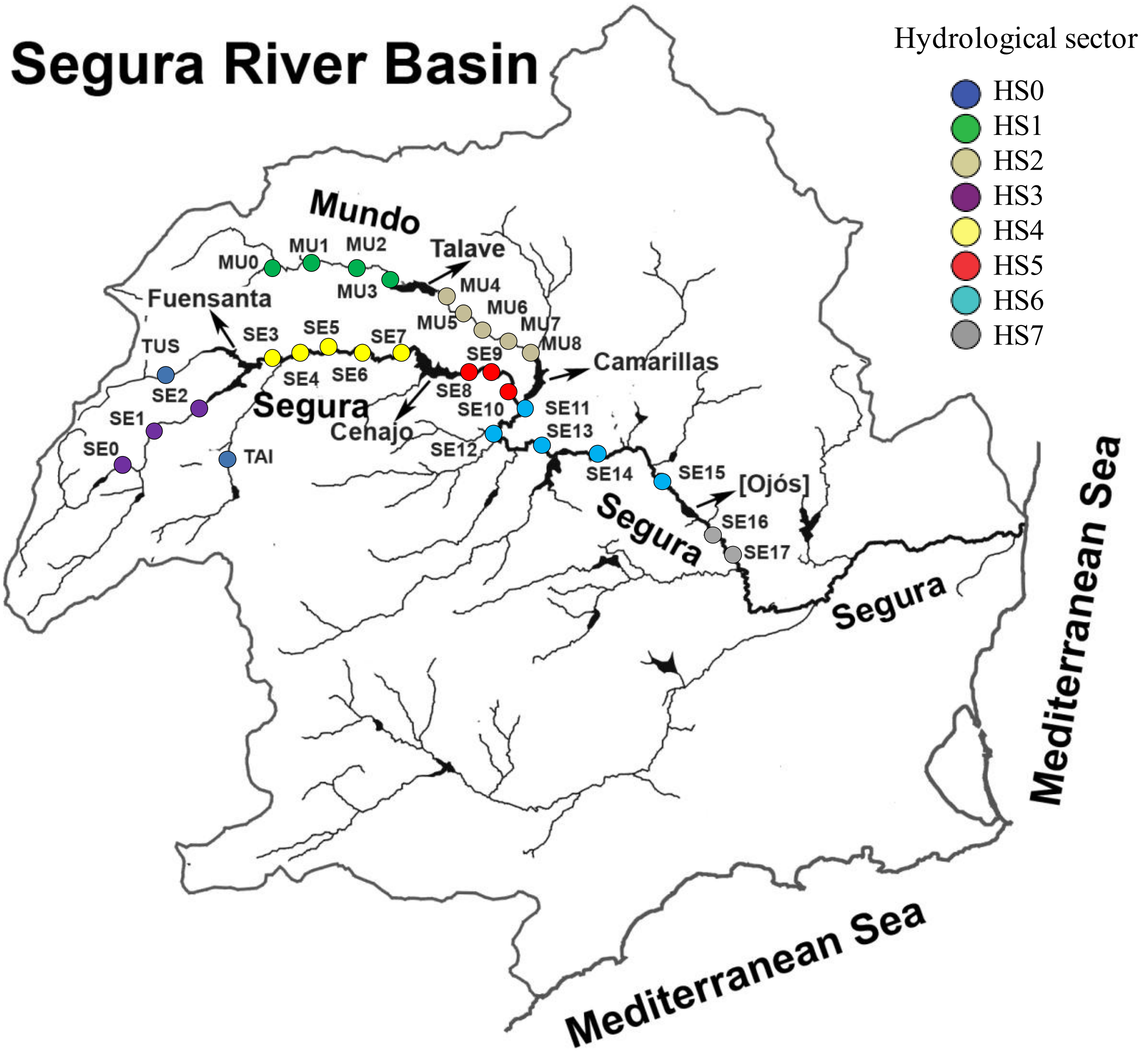

Figure 4.1. Sampling sites distributed along the Segura River basin. Hydrological sectors (HS0-HS7) are shown.

Other non-native species present in the study area are northern pike *Esox lucius* L., 1758, pike-perch *Sander lucioperca* (L., 1758), largemouth black bass *Micropterus salmoides* (Lacépède, 1802), common carp *Cyprinus carpio* L. 1758, Iberian straight-mouth nase *Pseudochondrostoma polylepis* (Steindachner, 1864), rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) and southern Iberian spined-loach *Cobitis paludica* (de Buen, 1930). Native species brown trout *Salmo trutta* L., 1758 and southern Iberian chub *Squalius pyrenaicus* (Günther, 1868) are only present in upper reaches (Oliva-Paterna *et al*., 2014) (see Chapter 2 for more information).

Field sampling and fish metrics

Specimens were captured by electrofishing during autumn 2010. Fish were collected with standard AC electrofishing equipment (1800W generator, working voltage between 200 and 350V, 2–3 A) following the procedure described in the *CEN Water Analysis-Fishing with electricity* (CEN, 2003). In order to obtain representative samples from each sampling site in relation to meso and microhabitats, a length of 100 meters was prospected. Existence of a high population connection between individuals caught in the same hydrological sector and a low connection between fish captured in adjacent sectors upstream was assumed. A total of 4825 specimens (1560 *L. sclateri*, 1280 *G. lozanoi*, 1636 *A. alburnus* and 349 *L. gibbosus*) were collected, counted and measured *in situ* (fork length L_F ± 1 mm and total body mass ± 0.1 g). Non-native individuals were sacrificed in an overdose solution of anaesthetic according with Spanish regulations and preserved in 10% buffer formaldehyde solution. Native individuals were returned to the river after the non-lethal anaesthetic effect.

Each species was assessed separately. Population structure was assessed at each sampling site, analysing length-frequency distributions compared both graphically and through 11 size-related variables: (1) mean ($L_F$mean, cm); (2) maximum ($L_F$max, cm); (3) minimum ($L_F$min, cm); (4) size amplitude (Range, cm); (5) variance ($L_F$var); (6) coefficient of variation ($L_F$cv); (7) skewness ($L_F$skew); (8) kurtosis ($L_F$kurt); (9) the 95th percentile (L_F95, cm) from the length-frequency distributions (1 or 0.5-cm class intervals); (10) number of size classes (NumSC, 1-cm intervals); and (11) size diversity (Size div), which was calculated for each population according to the nonparametric approach of Quintana *et al.* (2008). High values of size diversity reflect populations with similar proportions of size-classes, while lower values reveal high abundance of a particular size range (Quintana *et al.*, 2008). This parameter has been previously used in other studies of freshwater fish in relation with disturbance gradients in lakes (Emmrich *et al.*, 2011; Arranz *et al.*, 2015) and fish assemblages in rivers (Benejam *et al.*, 2015), but no studies about size diversity of fish populations have been developed in rivers (Benejam *et al.*, 2015). Finally, somatic condition (Kr) for each individual was estimated by the residuals from the least square regression of transformed weight on fork length (Sutton *et al.*, 2000). This residual index (Kr) avoids the effects of size variation and provides an appropriate alternative to the more traditional condition indexes (i.e. relative condition factor and Fulton’s condition factor).

Abiotic gradients

Each sampling site was characterised by the following 6 environmental variables: altitude (Alt) (meters above sea level), distance from the headwater source (DistSource) (Km), ecological status *sensu* EU Water Framework Directive (Status) (with the following categories: 1 = high; 2 = good; 3 = moderate; 4 = poor), conductivity (µS cm−1), Fluvial Habitat Index (IHF) and Riparian Quality Index (RQI) (Table 4.1). A principal component analysis (PCA) was performed (Hab_PCA) to obtain a gradient of sampling sites in relation with environmental variables.

Table 4.1. Environmental variables at each sampling site. Hydrological sector (HS0-HS7); Code of each sampling site; Type of stretch in each sampling site (1-3); Water temperature (Temp., ºC); Distance from the source of the Segura River (Dist. Source, Km); Ecological status (Status) (1-4); Altitude (m.a.s.l.); IHF and RQI indexes and Water conductivity (Cond., ±0.1 µs cm^{-1}).

Hydr. sector	Code	Type of stretch	Temp. (ºC)	Dist. Source (Km)	Status	Altitude (m.a.s.l.)	IHF	RQI	Cond. (µs cm^{-1})
HS0	TUS	1	14.5	25	1	809	84	65	432.33
	TAI	1	14.7	40	2	640	61	43	566.33
HS1	MU0	1	14.67	57	2	580	87.5	103	597.33
	MU1	1	14.67	60	2	560	72	55	602.33
	MU2	1	14.67	62.7	2	540	81	85	619.33
	MU3	3	14.67	65	2	520	81	85	619.33
HS2	MU4	1	14.72	74	3	480	69	43	639
	MU5	1	14.72	77.3	3	460	75	53	674.33
	MU6	2	14.72	81	3	430	70	64	687.67
	MU7	2	14.72	88	3	396	71.5	46	724.33
	MU8	3	14.72	101	3	354	70	34	1303.33
HS3	SE0	1	14.28	29.2	3	860	78	94	364
	SE1	1	14.28	46.4	1	685	61	98	386.67
	SE2	3	14.28	57.8	1	491	63	87	390.33
HS4	SE3	3	13.87	65	1	480	67	78	340
	SE4	1	13.87	75	1	470			
	SE5	1	13.87	93	1	460			
	SE6	1	13.87	102.9	1	452	68	80	401
	SE7	3	13.87	110	1	432	78	98	401.33
HS5	SE8	3	14.1	131	2	363	67	77	446.67
	SE9	1	14.1	140	2	325	73	66	491.67
	SE10	1	14.1	150	2	306	58	69	579.33
HS6	SE11	1	15.39	111	4	350	67	39	1108.67
	SE12	2	15.39	160.5	2	290	61.5	32	755.67
	SE13	2	15.69	174.7	3	260	57	44	803
	SE14	2	16.43	190	2	200	66	70	860.67
	SE15	1	16.43	209	2	148	58	45	1139.33
HS7	SE16	1	15.92	216	4	132			1102
	SE17	1	16.87	225	4	112	64	36	1157.33

Hydrological sector characterisation was carried out using mean daily flow data collected from gauging stations in each hydrological sector (CHS, 2017) and converted to a standard unit of measurement (m^3 s^{-1}). The hydrological period considered included the years 1994 to 2010. Hydrological metrics were calculated with the Time Series

Analysis TSA within the River Analysis Package (RAP version 3.0.7) (Marsh *et al.*, 2006). The following hydrological metrics were chosen as flow regime descriptors: MDBF (Mean Daily Base flow, m^3 s^{-1}), Fv (Flow variability, $Q_{90\%}$-$Q_{10\%}$), HSPeak (Mean of High Spell Peaks, m^3 s^{-1}), Contingency (based on monthly mean daily flow, understood as a periodicity measure between flows achieving higher values when the flow pattern is similar every year) and Predictability (based on monthly mean daily flow, understood as probability to predict a flow at a given moment). Hydrological sectors were distributed according to a flow regulation gradient obtained from a PCA with the flow metrics seen above and the average temperature (Reg_PCA) (Table 4.2). The natural thermal regime in freshwaters determines important ecological processes such as growth rates of aquatic organisms or nutrient cycling and productivity (Caissie, 2006; Webb *et al.*, 2008). Presence of regulation structures can alter this natural thermal regime of the water by selective releases of hypolimnetic (cold) or epilimnetic (warm) water from large reservoirs or by changes in frequency and timing of water temperature in small weirs (Olden & Naiman, 2010; Cai *et al.*, 2018). To assess the usefulness of both PCAs, the Kaiser-Meyer-Olkin's (KMO) measure of sampling adequacy was used (values above 0.5).

Table 4.2. Hydrological and temperature variables at each hydrological sector. Mean of High Spell Peaks (HSPeak, m^3s^{-1}); Flow variability (Fv, $Q_{90\%}$-$Q_{10\%}$); Mean Daily Base flow (MDBF, m^3s^{-1}); Predictability; Contingency and Water Temperature (Temp., ºC).

Sectors	Hydr. sectors	HSPeak	Fv	MDBF	Predictability	Contingency	Temp. (ºC)
Tributaries	HS0	10.647	-2.437	1.594	0.57	0.119	14.6
Above Talave	HS1	10.647	-2.437	1.594	0.57	0.119	14.67
Talave-Camarillas	HS2	29.487	-1.71	8.577	0.532	0.112	14.72
Above Fuensanta	HS3	29.495	-3.136	2.906	0.536	0.135	14.28
Fuensanta-Cenajo	HS4	17.531	-2.575	4.14	0.494	0.078	13.87
Down Cenajo-Juntas	HS5	23.794	-13.098	1.77	0.31	0.213	14.1
Juntas-Mulata	HS6	44.771	-1.621	14.035	0.573	0.107	15.39
Mulata-Andelma		43.946	-1.545	14.215	0.582	0.095	15.69
Andelma-Ojós		43.056	-1.467	15.141	0.587	0.088	16.43
Down Ojós	HS7	18.501	-3.312	2.461	0.474	0.117	16.87

Statistical analyses

The heterogeneity in the data and a possible bias produced by fishing catch methods, which could be underestimating the lower sizes, was avoided (Peterson *et al.*, 2004) by using only fish larger than 6 cm of fork length for *L. sclateri* and *A. alburnus* and 5 cm of fork length for *G. lozanoi* and *L. gibbosus* in the subsequent analyses.

Before analysis, all size-related variables were transformed [$\log_{10}(x+1)$]. In order to evaluate changes of these variables in relation with flow alteration, an analysis of covariance (ANCOVA) was performed for each species. Only sampling sites with more than 10 individuals and hydrological sectors with more than one sampling site were included in the analysis. Log-transformed size-related variables were used as dependent variables and the PC1 from the environmental PCA (Hab_PC1), which acts as a surrogate of the longitudinal gradient, was the covariate. The factors considered were: hydrological sectors (understood like as sampling sites sharing the same flow characteristics) and type of stretch (understood like as the different sampling sites see above, and categorized as: 1 = free-flow habitats, 2 = bypass artificial-flow habitats and 3 = lentic artificial-flow habitats) (Table 4.1). Models containing all explanatory variables (hydrological sector and type of stretch) and their interaction were considered significant when P value was 0.1.

Statistical analyses were performed with the SPSS® software package v. 15.0, and a significant level of 0.05 and marginal level of 0.1 was accepted.

Results

Abiotic gradients

The resulting PCA from environmental variables (Hab_PCA) of sampling sites explained 84.34% of the variation on axis 1 (Hab_PC1) and 15.09% on axis 2 (Hab_PC2). The high KMO (= 0.75) indicated the usefulness of the analysis. Hab_PC1 reflected the longitudinal gradient of the river according to land use and population density. Sites situated in higher altitudes showed greater values of IHF (Correlation coefficient $r = 0.55$; $P = 0.004$) and RQI ($r = 0.30$; $P = 0.133$), while downstream sampling sites displayed more conductivity ($r = -0.35$; $P = 0.077$) and worse ecological status ($r = -0.11$; $P = 0.592$) (Fig. 4.2A).

The PCA built with hydrological variables and mean temperature (Reg_PCA) showed a KMO = 0.51. The first axis (Reg_PC1) explained 92.81% of the variation and was mostly related to flow variability (Correlation coefficient $r = 0.96$; $P < 0.001$), contingency ($r = -0.93$; $P < 0.001$) and predictability ($r = 0.89$; $P < 0.001$). Negative scores correspond to similar inter-years flow patterns (high contingency values) and the positive scores mainly indicated river stretches with high daily flow variability but more predictable monthly flow. The second axis (Reg_PC2) explained 5.95% of the variation and it was positively related to the mean value of high spell peaks ($r = 0.99$; $P < 0.001$), and mean daily base flow ($r = 0.93$; $P < 0.001$). Hydrological sectors displaying natural flow conditions HS0 and HS1 (NATURAL) were located on the down right corner of the PCA graph (Fig. 4.2B). These sectors have an elevated seasonal flow variation, with high short-time flow peaks in the spring and autumn and severe droughts in summer (HS0 and HS1 in Fig. 4.3). From this natural flow sectors, the PCA distributed the rest of the hydrological sectors in two gradients. The extreme of one of them was situated on negative scores of Reg_PC1. It was called IMPACT 1 and HS5 was situated there. It showed high contingency and a reverse flow regime (non-natural), with high flow in summer and low flow in autumn (Fig. 4.2B and Fig. 4.3). The other extreme, placed on positive scores of both axes (Fig. 4.2B), grouped the sector HS6 that display high flow peaks and temperatures, as well as constant and high flows throughout the year (Fig. 4.3). It was called IMPACT 2.

Distribution of sampling sites following the habitat gradient was different when they were assessed with hydrological variables (Fig. 4.2).

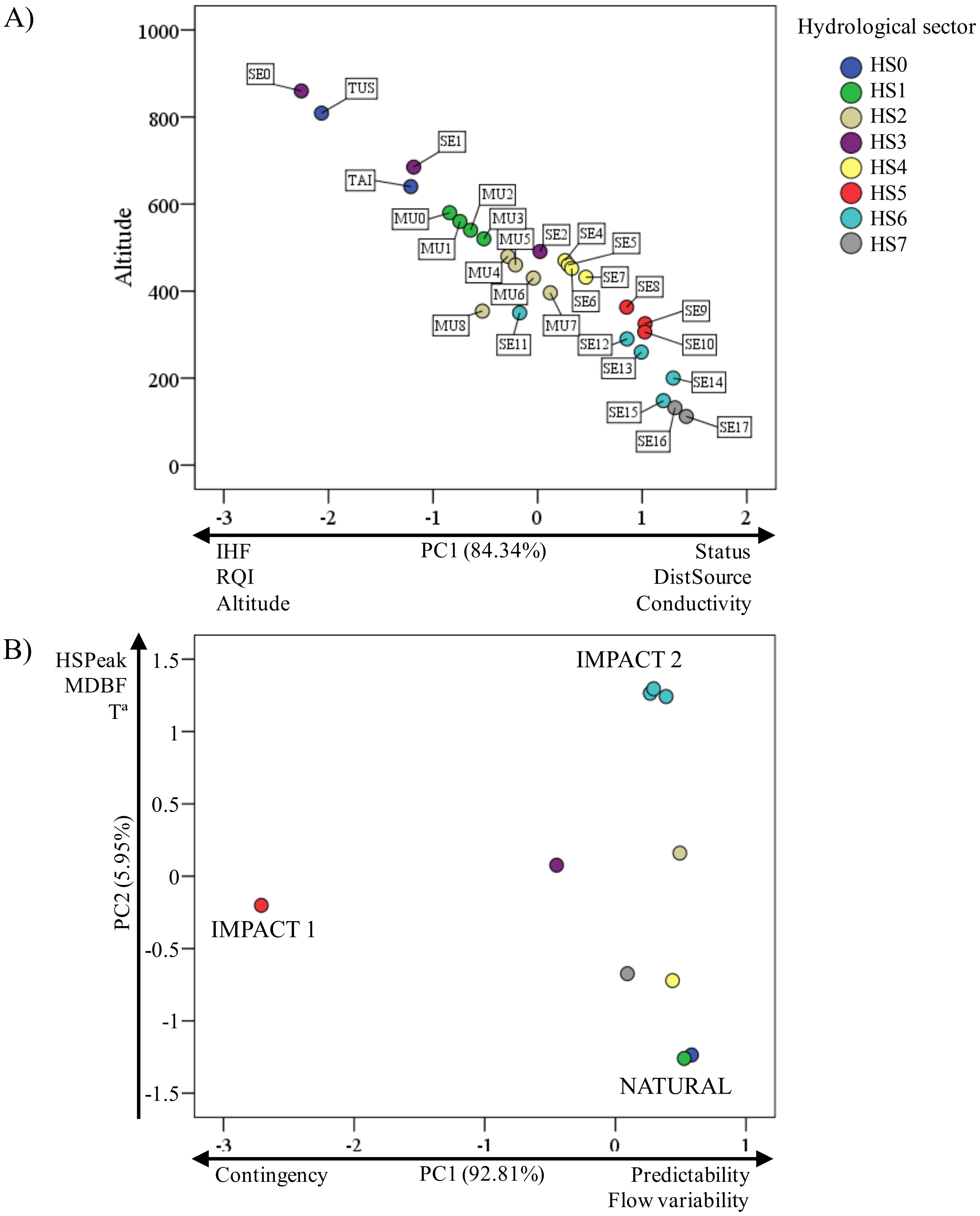

Figure 4.2. A) Sampling site distribution according the PC1 of habitat gradient and altitude. B) Allocation of hydrological sector based on PCs from flow metrics.

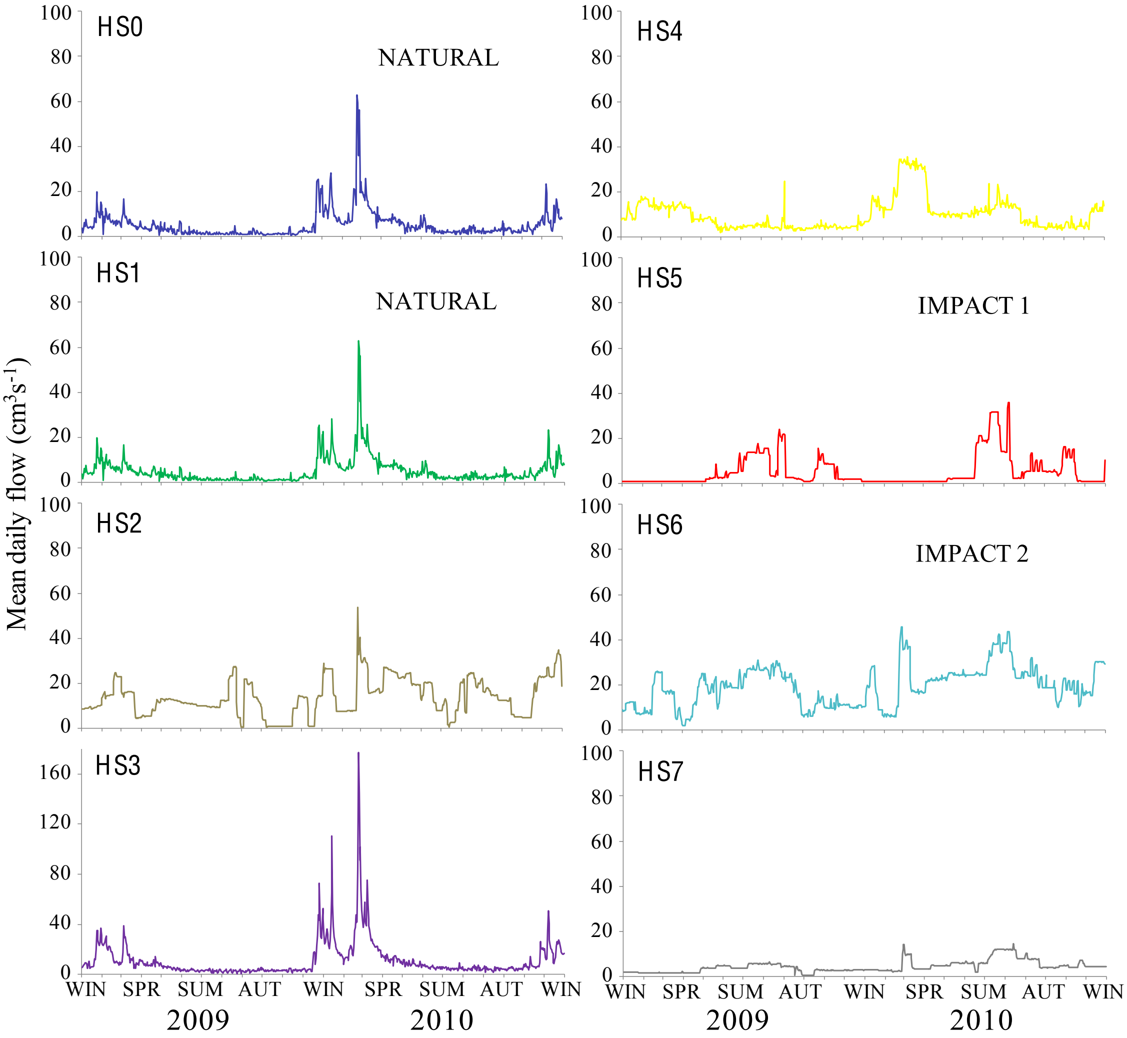

Figure 4.3. Mean daily flow (cm^3 s^{-1}) of each hydrological sector in the biennial cycle 2009-2010. Hydrological sectors with natural flow pattern and hydrological sectors catalogued as IMPACT 1 and 2 are shown.

Length-frequency distributions and fish metrics

Gobio lozanoi and *L. sclateri* were present in most sampling sites along the Segura River basin. *Lepomis gibbosus* and *A. alburnus* populations were not present above 540 m.a.s.l. and *L. gibbosus* was the species less abundant in the whole basin.

Luciobarbus sclateri populations showed wide size range in all hydrological sectors from 24 to 54.4 cm of amplitude. Their length-frequency distributions were characterised by predominance of small sizes (individuals between 5 and 20 cm L_F), being only HS7 the hydrological sector which did not follow that pattern (Fig. 4.4). HS6 also broke the general model because presented predominance of small sizes but together with a high number of medium (20-35 cm L_F) and large (more than 35 cm L_F)

individuals. In headwaters hydrological sectors (HS0 and HS3) no individual longer than 30 cm L_F was caught while individuals of 50-60 cm L_F were recorded in the rest of sectors (Fig. 4.4).

Gobio lozanoi presented populations with similar size amplitude in all hydrological sectors (from 7 to 10 cm L_F of amplitude) except HS7, which was narrower (5.2 cm of amplitude). Individuals between 5 and 9 cm L_F were predominant in the most of hydrological sectors except in HS6 where no small, medium or large individuals showed dominance (Fig. 4.4).

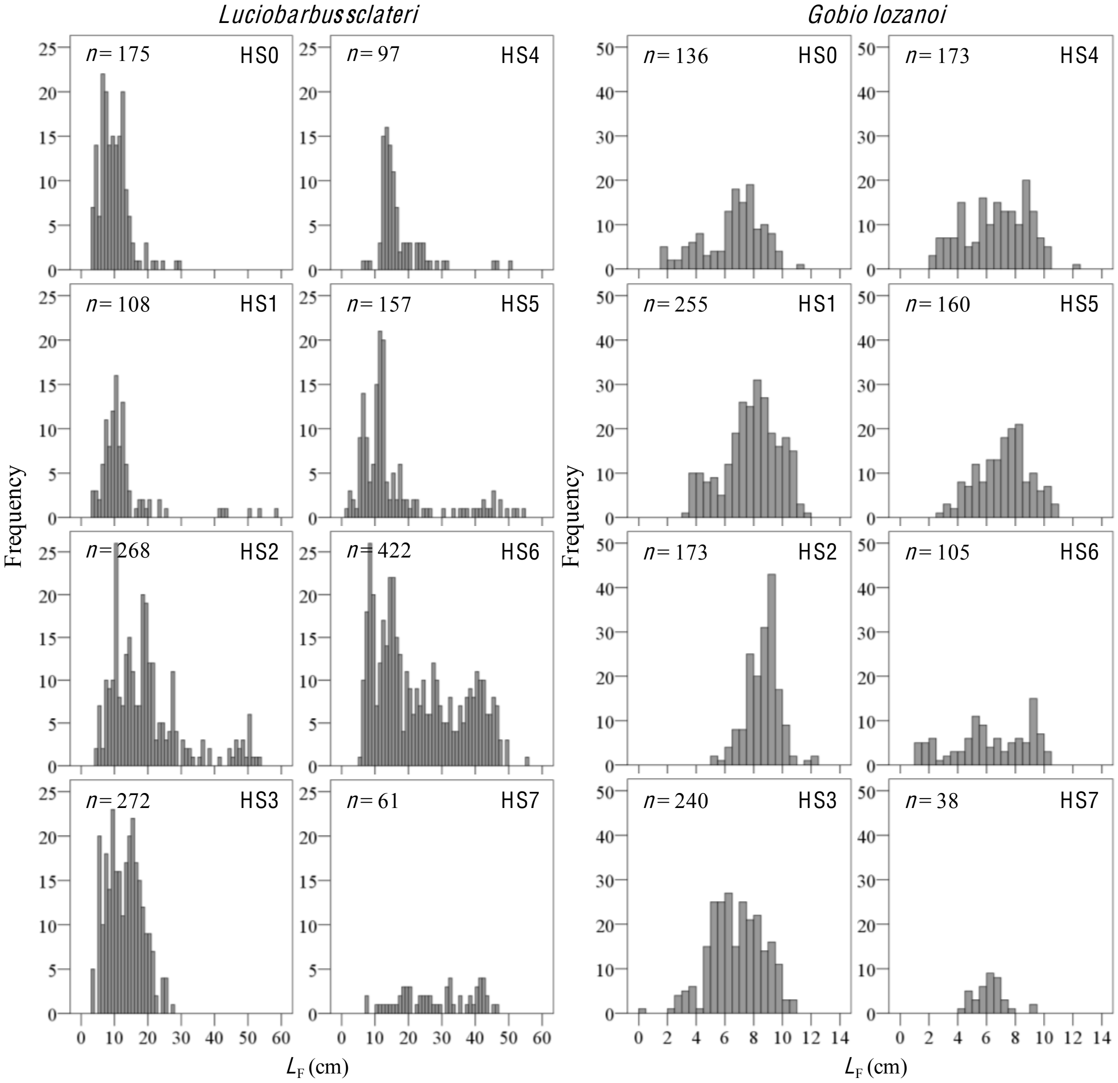

Figure 4.4. Length-frequency distributions of samples of *Luciobarbus sclateri* and *Gobio lozanoi* in each hydrological sector. Size intervals of 1 cm and 0.5 cm in *L. sclateri* and *G. lozanoi*, respectively (*n*, number of individuals).

Length-frequency distributions of *L. gibbosus* and *A. alburnus* populations showed a high variability among hydrological sectors and no general patterns were observed. *Alburnus alburnus* populations clearly presented bimodal length-frequency distributions with predominance of small sizes (4-7 cm L_F) in HS7, medium sizes (7-11 cm L_F) in HS4 and large sizes (11-16 cm L_F) in HS2 and HS6 (Fig. 4.5). *Lepomis gibbosus* showed the widest length-frequency distributions in the hydrological sectors HS2 and HS6 where, additionally, large sizes were predominant (8-10 cm L_F) (Fig. 4.5).

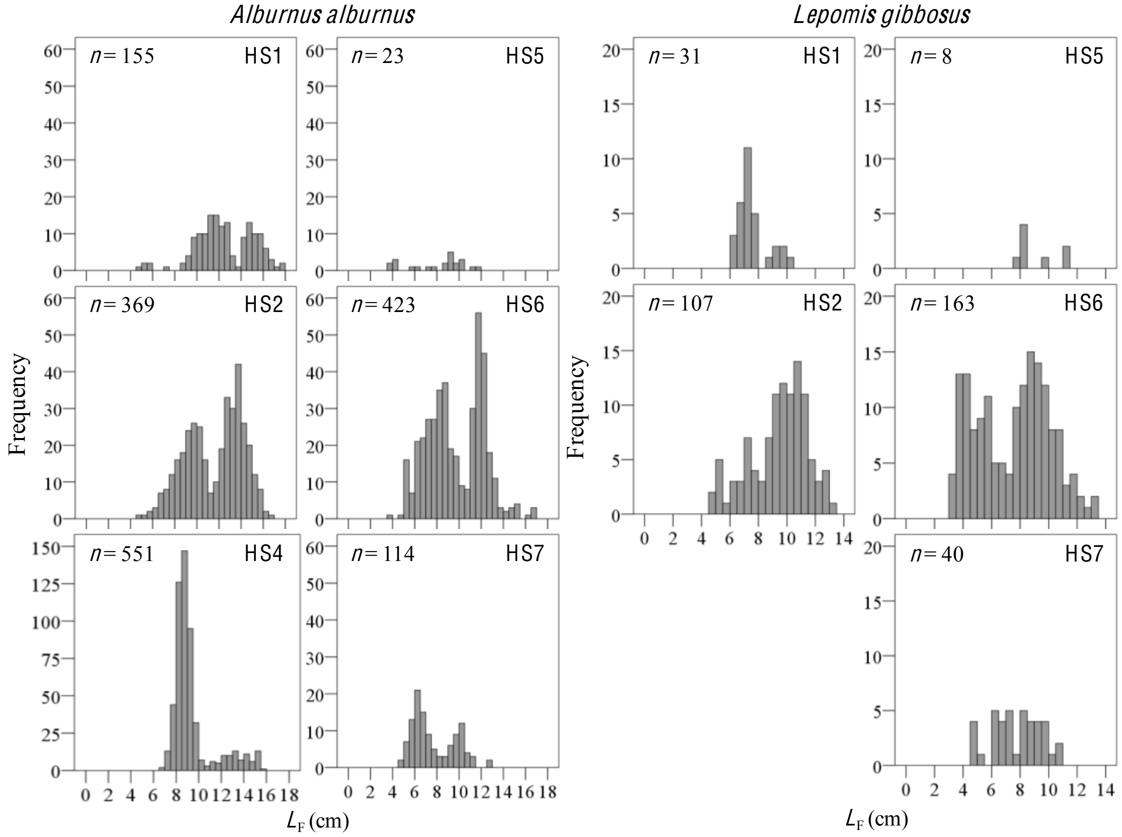

Figure 4.5. Length-frequency distributions of samples of *Alburnus alburnus* and *Lepomis gibbosus* in each hydrological sector. Size intervals of 0.5 cm (*n*, number of individuals).

ANCOVA results showed that flow alteration and type of stretch have been significant factors affecting size-related variables of populations (Table 4.3). Size-related variables of all species had some significant interactions with the model except for *A. alburnus*, where only somatic condition showed significant interactions (Table 4.3). *Luciobarbus sclateri* size-related variables were mostly influenced by flow characteristics of the different hydrological sectors. However, mean and minimum fork length were also influenced by type of stretch and size amplitude, number of size classes, size diversity and somatic condition by the interaction of both factors (hydrological sector x type of stretch). On the other hand, *L. gibbosus* size-related variables were influenced by type of stretch, although maximum fork length, size amplitude, number of size classes and size diversity showed significance with all factors. Length variance, coefficient of variation, number of size classes and size diversity of *G. lozanoi* turned out to be affected by the factors interaction, even though some size-related variables and somatic condition were also influenced by the single factors (Table 4.3).

Table 4.3. Results of ANCOVA analyses of the size-related variables and somatic condition (Kr) of target species along the Segura River basin. Hab_CP1 is the covariate and hydrological sectors (HS) and type of stretch (TS) are the factors. Mean length ($L_F$mean), maximum length ($L_F$max), minimum length ($L_F$min), size amplitude (Range), variance ($L_F$var), coefficient of variation ($L_F$cv), 95th percentile (L_F95), skewness ($L_F$skew), kurtosis ($L_F$kurt), number of size classes (NumSC), size diversity (Size div) and somatic condition (Kr). Significant results in bold (*P* value < 0.1).

Response		*Gobio lozanoi*			*Luciobarbus sclateri*			*Lepomis gibbosus*			*Alburnus alburnus*		
variable	Model	df	*F*	*P* value	df	*F*	*P* value	df	*F*	*P* value	df	*F*	*P* value
$L_F$mean													
	Hab_CP1	1	0.879	0.376	1	0.344	0.571	1	2.517	0.173	1	0.302	0.606
1	HS	5	3.705	**0.049**	5	7.180	**0.004**	2	2.096	0.218	2	1.695	0.274
2	TS	2	4.817	**0.042**	2	4.515	**0.040**	2	3.241	0.125	2	0.049	0.952
3	HS x TS	5	3.242	**0.068**	5	2.347	0.118	1	3.327	0.128	2	0.391	0.696
$L_F$max													
	Hab_CP1	1	2.059	0.189	1	2.729	0.130	1	312.769	**<0.001**	1	3.211	0.133
1	HS	5	1.280	0.359	5	1.603	0.245	2	121.842	**<0.001**	2	1.188	0.378
2	TS	2	3.587	**0.077**	2	0.136	0.875	2	141.923	**<0.001**	2	1.390	0.331
3	HS x TS	5	1.510	0.288	5	2.503	0.102	1	52.190	**0.001**	2	0.427	0.674
$L_F$min													
	Hab_CP1	1	0.310	0.593	1	3.517	**0.090**	1	0.119	0.744	1	0.004	0.950
1	HS	5	1.349	0.336	5	6.609	**0.006**	2	0.0401	0.690	2	0.249	0.789
2	TS	2	1.015	0.405	2	6.933	**0.013**	2	4.118	**0.088**	2	0.653	0.560
3	HS x TS	5	1.461	0.301	5	6.219	**0.007**	1	0.391	0.559	2	0.813	0.495
Range													
	Hab_CP1	1	3.002	0.121	1	2.877	0.121	1	54.305	**0.001**	1	2.015	0.215
1	HS	5	1.227	0.379	5	1.598	0.247	2	19.426	**<0.001**	2	0.371	0.708
2	TS	2	2.013	0.196	2	0.168	0.847	2	29.953	**0.004**	2	2.207	0.206
3	HS x TS	5	2.376	0.133	5	3.224	**0.054**	1	17.533	**0.009**	2	0.192	0.831
$L_F$var													
	Hab_CP1	1	0.054	0.822	1	1.333	0.275	1	14.530	**0.012**	1	1.539	0.270
1	HS	5	0.420	0.823	5	2.083	0.151	2	1.928	0.240	2	0.348	0.722
2	TS	2	0.278	0.764	2	0.046	0.955	2	7.466	**0.032**	2	1.054	0.415
3	HS x TS	5	3.225	**0.069**	5	1.859	0.189	1	4.632	**0.084**	2	0.766	0.513
$L_F$cv													
	Hab_CP1	1	0.001	0.972	1	1.443	0.257	1	9.811	**0.026**	1	0.876	0.392
1	HS	5	0.690	0.645	5	2.914	**0.071**	2	1.718	0.270	2	0.271	0.773
2	TS	2	1.430	0.294	2	1.199	0.341	2	4.672	**0.072**	2	1.011	0.428
3	HS x TS	5	3.862	**0.045**	5	1.272	0.348	1	2.114	0.206	2	0.541	0.613

Table 4.3. Continuation

Response			*Gobio lozanoi*			*Luciobarbus sclateri*			*Lepomis gibbosus*			*Alburnus alburnus*	
variable	Model	df	*F*	*P* value	df	*F*	*P* value	df	*F*	*P* value	df	*F*	*P* value
L_F95													
	Hab_CP1	1	0.108	0.752	1	2.035	0.187	1	--	--	1	2.888	0.164
1	HS	5	1.482	0.306	5	1.658	0.240	2	--	--	2	2.424	0.204
2	TS	2	4.184	**0.064**	2	0.120	0.889	2	--	--	2	0.502	0.639
3	HS x TS	5	0.333	0.802	5	1.998	0.179	1	--	--	2	0.768	0.522
$L_F$skew													
	Hab_CP1	1	0.165	0.697	1	0.341	0.572	1	0.690	0.453	1	1.122	0.349
1	HS	5	1.647	0.264	5	2.159	0.141	2	0.529	0.262	2	1.989	0.251
2	TS	2	2.100	0.193	2	1.408	0.289	2	0.700	0.549	2	0.612	0.586
3	HS x TS	5	2.271	0.157	5	0.820	0.562	1	0.000	0.988	2	0.051	0.833
$L_F$kurt													
	Hab_CP1	1	0.799	0.406	1	0.124	0.736	1	2.135	0.240	1	1.231	0.348
1	HS	5	1.874	0.233	5	3.392	**0.085**	2	2.427	0.236	2	0.647	0.584
2	TS	2	3.597	**0.094**	2	2.581	0.155	2	2.714	0.212	2	0.115	0.895
3	HS x TS	5	0.842	0.565	5	1.153	0.426	1	2.555	0.208	2	0.192	0.835
NumSC													
	Hab_CP1	1	0.026	0.875	1	0.816	0.387	1	21.111	**0.006**	1	0.986	0.366
1	HS	5	3.454	**0.059**	5	2.921	**0.070**	2	7.202	**0.034**	2	0.223	0.807
2	TS	2	1.195	0.352	2	2.205	0.161	2	7.610	**0.030**	2	1.697	0.274
3	HS x TS	5	6.709	**0.010**	5	7.143	**0.004**	1	11.575	**0.019**	2	0.234	0.800
Size div													
	Hab_CP1	1	0.828	0.390	1	0.742	0.409	1	20.816	**0.006**	1	0.047	0.837
1	HS	5	1.919	0.197	5	3.817	**0.034**	2	8.177	**0.027**	2	0.019	0.981
2	TS	2	2.456	0.147	2	1.027	0.393	2	10.231	**0.017**	2	0.886	0.468
3	HS x TS	5	3.105	**0.075**	5	2.579	**0.095**	1	6.713	**0.049**	2	2.424	0.184
Kr													
	Hab_CP1	1	0.920	0.365	1	3.102	0.109	1	1.683	0.251	1	5.314	**0.069**
1	HS	5	8.634	**0.004**	5	3.627	**0.039**	2	1.758	0.264	2	4.183	**0.086**
2	TS	2	10.345	**0.006**	2	1.696	0.232	2	0.244	0.792	2	2.902	0.146
3	HS x TS	5	2.145	0.161	5	3.184	**0.056**	1	1.918	0.225	2	4.106	**0.088**

Four size-related variables ($L_F$mean, $L_F$max, $L_F$skew and Size div) were selected as relevant variables explaining size structure of populations. The mean values of these size-related variables and somatic condition were studied among hydrological sectors and types of stretches (Fig. 4.6 and Fig. 4.7). Depending on species, the five variables behaved differently. For example, $L_F$mean of *L. sclateri* incremented along hydrological sectors (ordered in the graph following the longitudinal gradient of the river), while $L_F$mean of *A. Alburnus* decreased; however, not clear pattern was observed in $L_F$mean of *G. lozanoi* and *L. gibbosus*. Only *L. sclateri* presented relevant differences in $L_F$max with shorter maximum length in two headwater hydrological sectors (HS0 and HS3). High values of $L_F$skew reflect a population structure conformed by elevated number of small individuals and presence of few large individuals (i.e. *L. sclateri* in HS1 and HS4

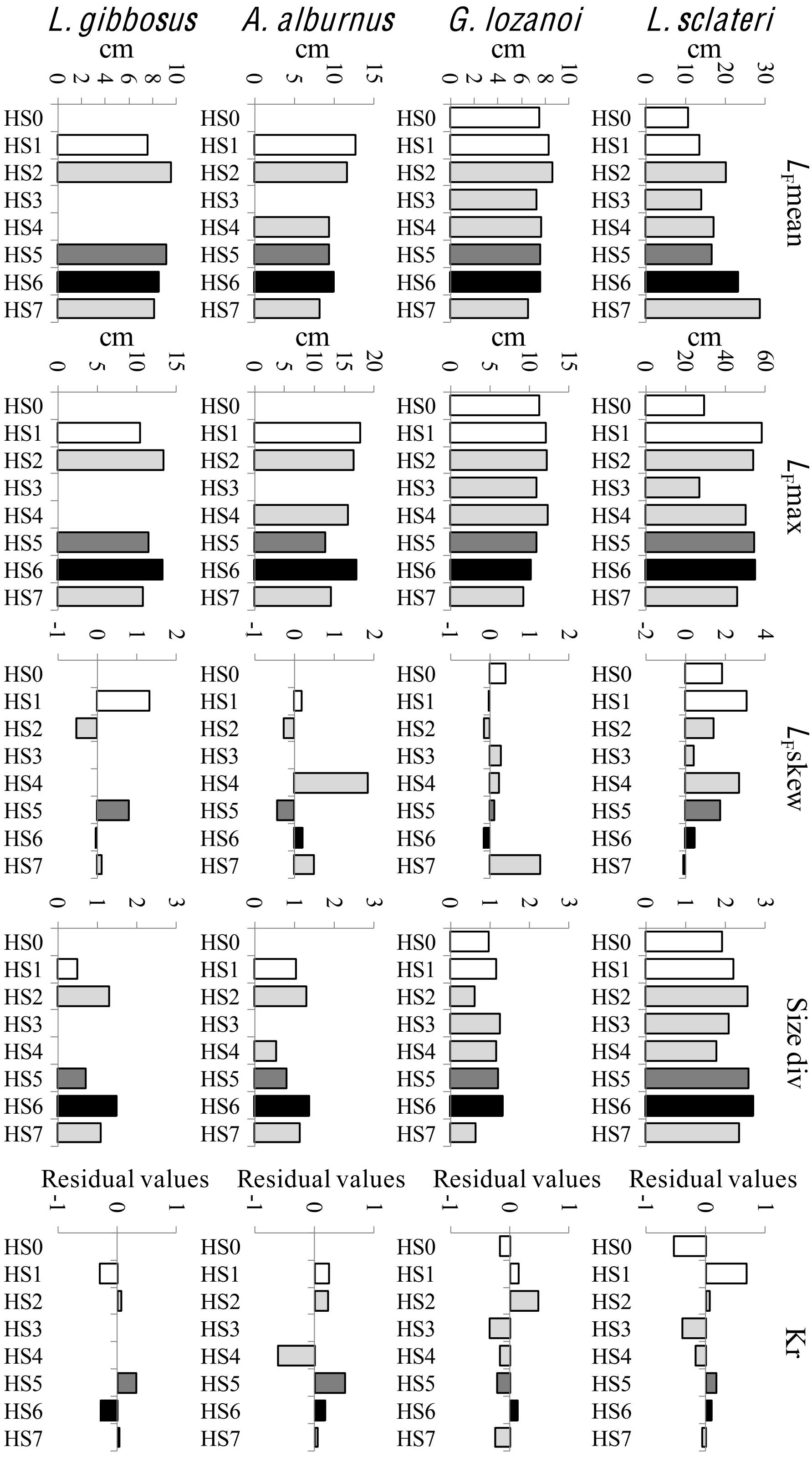

Figure 4.6. Mean values of four size-related variables: mean length ($L_F$mean), maximum length ($L_F$max), skewness ($L_F$skew), size diversity (Size div) and somatic condition (Kr) by hydrological sectors (HS0-HS7) for each target species. White bars represent NATURAL hydrological sectors (HS0 and HS1), dark grey bars represent IMPACT 1 hydrological sector (HS5) and black bars represent IMPACT 2 hydrological sector (HS6).

in Fig. 4.4). Size diversity resulted higher in IMPACT 2 than in NATURAL hydrological sectors. Tributary rivers (HS0) did not seem to favour somatic condition of rheophilic fish, while HS1 and HS6 presented populations with good somatic conditions in all target species with the exception of *L. gibbosus*. IMPACT 1 hydrological sector (HS5) presented the exotic species populations (*L. gibbosus* and *A. Alburnus*) with higher somatic conditions (Fig. 4.6).

The variables above considered were also influenced by type of stretch (Table 4.3 and Fig. 4.7). Larger $L_F$mean were detected in lentic artificial-flow habitats in *G. lozanoi* and *A. Alburnus* populations, while *L. sclateri* and *L. gibbosus* populations showed higher values of $L_F$mean in bypass artificial-flow habitats. Lentic artificial-flow habitats and free-flow habitats were the types of stretches with largest sizes in all target species. Populations with high number of small individuals and scarce large ones (high $L_F$skew) were present in lentic artificial-flow habitats for *L. sclateri* and *L. gibbosus* populations and in free-flow habitats for the rest of species. Bypass artificial-flow habitats seemed to favour a higher size diversity of *L. sclateri* and *L. gibbosus* populations, while *G. lozanoi* populations were more diverse in free-flow habitats. Somatic condition of populations of *L. sclateri* and *G. lozanoi* showed the same pattern, being favoured by lentic artificial-flow habitats. However, *A. alburnus* and *L. gibbosus* showed inverse pattern of somatic condition in the types of stretches.

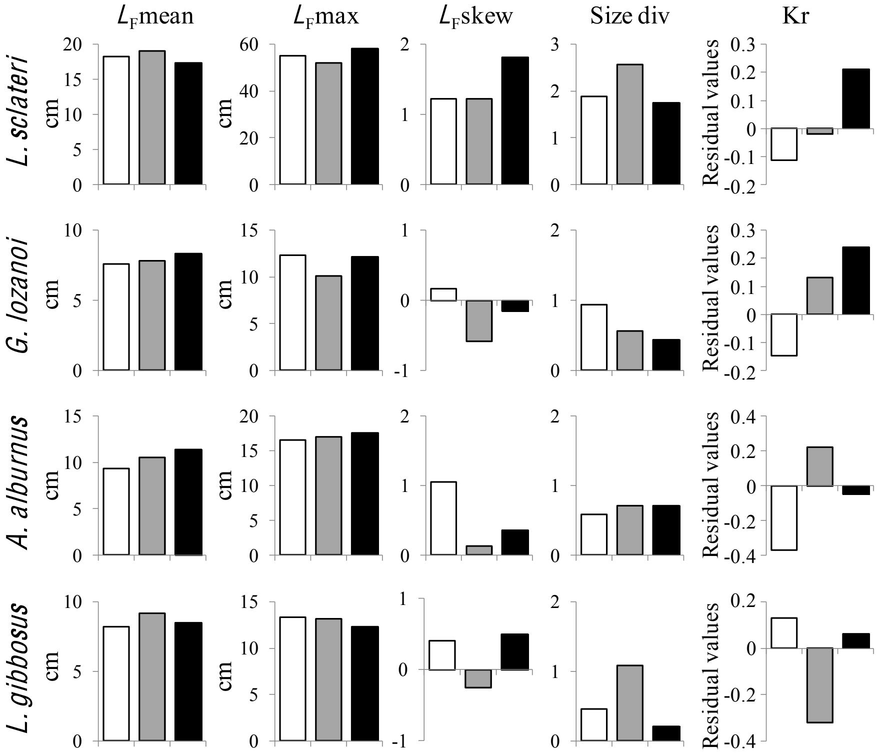

Figure 4.7. Mean values of four size-related variables: mean length ($L_F$mean), maximum length ($L_F$max), skewness ($L_F$skew), size diversity (Size div) and somatic condition (Kr) by types of stretches (1-3) for each target species. White bars represent free-flow habitats (1), grey bars represent bypass artificial-flow habitats (2) and black bars represent lentic artificial-flow habitats (3).

Discussion

Abiotic gradients

Distribution of sampling sites along the environmental longitudinal gradient concurs with human density and land use gradients displayed in the Segura River basin (Mellado, 2005). Sampling sites from headwaters streams located in forested areas sparsely populated showed higher values of IHF and RQI. Meanwhile, worse ecological status and high conductivity were found in sampling sites placed in the middle part of the basin, where there are intermediate population density and intensive agricultural land use (Mellado, 2005).

This longitudinal gradient is not followed when flow parameters are assessed. Results showed different flow patterns in the hydrological sectors encouraged by impoundment structures. Hydrological sectors representing NATURAL flow condition (HS0 and HS1) were located in headwaters and were characterised by a typical Mediterranean flow regime, with notable seasonal flow pattern with high short-time flow peaks in spring and autumn and severe droughts in summer. No impoundment structures exist in these areas and flow regime practically depends of the rainfall (Belmar-Díaz *et al.*, 2011). The hydrological sector called IMPACT 1 was located in the middle section of the Segura River (HS5) and it presented a reverse flow regime with high flow kept during the summer and very low flow in spring and autumn. Moreover, it showed an elevated contingency, which means that the flow pattern is similar every year. This particular flow regime is due to its localization, downstream the largest reservoir in the Segura basin, Cenajo reservoir, whose main uses are flood control and water supply for irrigation (CHS, 2017). Water demands for agricultural uses are higher in summer when natural rainfall and runoff are scarce, generating water discharge during the dry season, which produces inversion of the hydrological regime (Piqué *et al.*, 2016). Downstream IMPACT 1 was located the hydrological sector (HS6) considered IMPACT 2. It showed a reverse flow regime, elevated flow throughout the year and high daily flow variability. Reverse regime is due to its location downstream Cenajo and Camarillas (also used to flood control and to supply agricultural uses) reservoirs. Moreover, it is placed downstream Segura and Mundo rivers confluence, which supposes and increment of the mean base daily flow (Belmar-Díaz *et al.*, 2011). The high daily flow variability and unpredictable flow peaks are due to the presence of numerous bypass weirs for daily water diversions to agricultural and hydroelectric power use. The presence of artificial structures (dams or weirs) has an impact on the hydrology of the rivers (Bunn & Arthington, 2002; Poff *et al.*, 2007) and has an effect on monthly flows and flood magnitude and frequency at different temporal scales (daily, monthly and annually) in the Segura River basin (Piqué *et al.*, 2016).

Fish metrics and environmental guilds

Natural seasonal flow with sudden flash floods seems to be avoiding the successful establishment of the exotic species *A. alburnus* and *L. gibbosus,* which were only present close to the reservoir in the NATURAL area (MU3 in HS1), where the effect of natural flow is buffered (Bernardo *et al.*, 2003; Vinyoles *et al.*, 2007; Khedkar *et al.*, 2014). Absence of impoundment structures allows the seasonal Mediterranean flow regime, which does not favour ecological requirements of those species (Scott & Crossman, 1973; Haberlehner, 1988; Vinyoles *et al.*, 2007) (see Chapter 2 for more information). *Alburnus alburnus* showed higher mean fork length in this area, but it was due to a greater presence of large individuals than small ones. *Lepomis gibbosus* showed populations with narrow range of sizes and low somatic condition in the NATURAL area, possibly because of the high survival energetic costs to support the intense floods, which can carry on lower growth rates, recruitment failures, and inferior somatic condition (Ribeiro & Collares-Pereira, 2010). No-natural high flow peaks from Cenajo reservoir in the IMPACT 1 area is also avoiding the presence of those exotic species, which were scarce and caugth in the farthest sampling site downstream from the dam. However, they showed high values of somatic condition, perhaps because only stronger individuals were able to surpass drag effect of intense flows.

On the other hand, the rheophilic species *G. lozanoi* and *L. sclateri* presented individuals in all sampling sites of the hydrological sectors NATURAL and IMPACT 1. Species display morphological or behavioural adaptation to overtake environmental constraints of their native area (Minckley & Meffe, 1987; Lytle & Poff, 2004). The translocated *G. lozanoi* showed similar population structure in both areas (NATURAL and IMPACT 1) with presence of all size classes and predominance of median sizes. It also presented higher mean and maximum fork length in NATURAL area. Native of mountain streams, *G. lozanoi* is adapted to environments with moderate current (Doadrio & Madeira, 2004; Doadrio *et al.*, 2011) and it is expected to have the ability to withstand such disturbances and to avoid the drag effect of flash floods (Minckley & Meffe, 1987). The native species *L. sclateri* showed similar population structure in both areas in relation with the predominance of small sizes, but the presence of larger individuals were different. Headwaters geomorphological structure with numerous riffle areas with fast flow, smaller river width and cooler temperatures (Poff *et al.*, 1997) could have influence on the maximum growth of individuals (Weatherley & Gill, 1987; Lytle & Poff, 2004). Furthermore, slower growth rates and disappearance of large individuals have been related with low flows in other species (Xu *et al.* 2010; Merciai *et al.* 2017). Individuals from NATURAL area have to search for suitable refuges during severe summer conditions (Alexandre *et al.*, 2016) and the absence of deep pools in this area, which are more suitable as fish refuge and for presence of larger fish, contributed to find populations with poor somatic condition in tributary rivers. Although it is the native species of the basin, its optimal environmental requirements are found in the middle part of the river, where more habitat diversity allows them to find suitable areas for each life stage (Encina *et al.*, 2006; Oliva-Paterna *et al.*, 2003).

All species were present in the IMPACT 2 area. The altered flow and the existence of artificial-flow habitats consequence of impoundment structures, which could be working as potential refuges, have provided a suitable framework for the establishment of generalist and tolerant species (Bunn & Arthington, 2002; Mims & Olden, 2013). In fact all species showed higher size diversity, which indicate populations with wide size amplitude and representation of all size classes. However, the high base flow and flow variability could be negatively affecting *L. gibbosus* populations, which presented lower somatic conditions. Populations of *L. sclateri* showed higher presence of larger individuals in this area in comparison with the other hydrological sectors. Possibly, presence of more pools derived of weirs allows a continuous occurrence of individuals along the river (Benejam *et al.* 2014; Alexandre *et al.*, 2014a, 2016).

The presence of artificial structures in aquatic environments can alter the natural functionality of rivers (Richter *et al.*, 1996; Vörömarty *et al.*, 2003). In fact, flow pattern can be deeply modified by the presence of impoundment structures (reservoirs, dams, weirs), losing flow seasonality and natural disturbances, changing magnitude and frequency of flow and generating new suboptimal habitats, which could be occupied for more generalist faunas (Bunn & Arthington, 2002; Poff *et al.*, 2007; Mims & Olden, 2013). In this study, the existence of different types of stretches with presence or absence of artificial-flow habitat has also affected on size-related variables and population structure of target species.

Alburnus alburnus was the only species whose size-related variables were not directly affected by hydrological sectors or types of stretches. This species has demonstrated wide inter-population phenotypic plasticity in native areas (Biró & Muskó, 1995) and during the invasion process (Almeida *et al.,* 2014, 2017; Latorre *et al.*, 2016; Masó *et al.*, 2016), establishing viable populations in both lentic and lotic environments (Vinyoles *et al.*, 2007; Almeida *et al.,* 2014; Masó *et al.*, 2016). According to Vinyoles *et al.* (2007), reservoirs could be acting as refuge and source of individuals to river stretches, because the species was more present in river areas between large reservoirs. In fact, this suprapelagic species showed larger individuals and populations with better somatic conditions in more lentic areas (reservoirs or deep pools), where more stable hydrological conditions allow lower energy investment for swimming (Poff *et al.*, 1997) and major food resources availability exists (zooplankton production) (Akopian *et al.*, 1999), which is expected in areas more similar to its native habitat (Freyhof & Kottelat, 2008). However, population structure found in this study confirms the viability of *A. alburnus* populations far from reservoirs. Positive skewness of population size structure found in free-flow habitat sampling sites indicate a high presence of small-medium sizes, which suggests recruitment processes and/or a lack of predation pressure, which is more intense close to reservoirs (Torralva *et al.*, 2005; Andreu-Soler *et al.*, 2006). Predation is an important factor shaping the size structure of lake fish assemblages

(Jackson *et al.*, 2001; Emmrich *et al.*, 2011). Furthermore, worse somatic condition populations were found in free-flow habitats sampling sites where environmental characteristics were more different to its native requirements. No significant influences of flow and types of stretches on size-related variables of *A. alburnus* could be related to its wide phenotypic plasticity and other factors, such as its residence time in new invaded areas or mesohabitat characteristics not deal with in this study (see Chapter 3 for more information).

Presence of artificial-flow habitats seems to be a decisive factor for the success of *L. gibbosus* in the Segura River basin. In hydrological sectors with presence of bypass artificial-flow habitats *L. gibbosus* populations showed the widest size amplitude, greatest fork length and size diversity. Presence of water withdrawal structures provides lentic areas upwater of weirs and low flow downstream them, creating artificial-flow habitats more suitable for the development of this species typical of lentic waters (Scott & Crossman, 1973; Copp & Fox, 2007). Moreover, in free-flow sampling sites and sampling sites further from reservoirs, population structure showed more variability, which decreased as flow conditions were more altered, coinciding with this species is more abundant in altered areas and artificial systems (Almeida *et al.*, 2009; Ribeiro & Collares-Pereira, 2010). Lack of seasonal floods and continuous flow during the rainless summer have also encouraged establishment of *L. gibbosus* populations in other Iberian basins (Almeida *et al.*, 2009; Ribeiro & Collares-Pereira, 2010). Species of the genus *Lepomis* have been described as having a mix of equilibrium and periodic life-history strategies in the triangular model of life history evolution proposed by Winemiller & Rose (1992). The elevated representation of small sizes in the IMPACT 2 area, result of a higher recruitment beneficiated by the presence of artificial-flow habitat and absence of flash floods, could be suggested an equilibrium life-history strategy with a high survival of YOYs, displayed for the species in lentic areas in its native range (Fox *et al.*, 2007). However, the species has also exhibited high levels of phenotypic plasticity (Tomeček *et al.*, 2007) and the ability of changing its growth rates and reproductive traits in response to environmental conditions (Ribeiro & Collares-Pereira, 2010), showing life patterns associated with periodic-opportunistic life-history strategies in invaded areas (Vila-Gispert & Moreno-Amich, 2002; Copp & Fox, 2007; Fox *et al.*, 2007).

Populations of the rheophilic species *G. lozanoi* inhabiting free-flow habitats showed larger individuals, higher size diversity and high skewness, which indicates greater recruitment. These results would be the expected according with its native habitat requirements (Doadrio & Madeira, 2004; Doadrio *et al.*, 2011). However, its opportunistic life-history strategy (Vila-Gispert & Moreno-Amich, 2002) and its plasticity in life-history traits (Grossman & Sostoa, 1994; Lobón-Cervia *et al.*, 1991; Oscoz *et al.*, 2003) allow it to colonize a wide range of new habitats, which justify its extensive distributions and presence of successful populations in diverse aquatic environments in the Segura River basin (Andreu-Soler *et al.*, 2006; Amat-Trigo *et al.*, 2013) (see Chapter 2 for more information). In sampling sites associated with bypass artificial-flow habitat, populations did not show predominance of any size class, but such type of impoundment structure may be harboring small individuals. This result suggests a successful recruitment downstream the weirs and a suitable habitat structure with shallow and more erodible substrate, which could favour the development of juveniles of *G. lozanoi* (Grossman *et al.*, 1987; Grossman & Sostoa, 1994).

The rheophilic native species *L. sclateri* showed an increment of fork length along the longitudinal gradient, which could be related to higher rates of growth in regulated areas or elevated presence of larger individuals (Torralva *et al.*, 1997). Increment of mean fork length in bypass artificial-flow habitats was due to elevated presence of longer fish, less abundant in other types of stretches. Water withdrawal provides more pools and less riffle areas, and individuals can be abundant and keep a continuous distribution along the river section (Benejam *et al.* 2014; Alexandre *et al.*, 2014a, 2016). Moreover, abundant presence of small individuals suggests successfull recruitment downstream the weirs, where the reproduction event must be taking place due to the inability of the species to exceed the artificial barrier (Lucas & Frear, 1997; Vasconcelos *et al.*, 2014) and to suppose a suitable habitat to harbor juveniles of the species. Somatic condition of native *L. sclateri* was very variable among hydrological sectors and sampling sites. In the Segura River, fish condition of *L. sclateri* was mainly dependent on the ecological variables, good population conditions being favoured by continuous water flow, greater substrate diameter and presence of fish refuge (Oliva-Paterna *et al.*, 2003). In this study, in NATURAL area, somatic condition of populations was low. Other populations of the same species also showed low somatic conditions in autumn in non-regulated areas (Herrera & Fernández-Delgado, 1992; Torralva *et al.*, 1997). A more stressful flow framework, with seasonal peaks could lead to a lower somatic condition due to the acquisition of a more swim-fitted morphology to deal with the high flows (Alexandre *et al.*, 2014a,b). In addition, elevated oxygen concentration associated with constant higher flows and better riparian cover, which provide more food sources and refuges (Cowx & Welcomme, 1998), have been considered river characteristics favouring somatic condition of fish populations (Vila-Gispert *et al.*, 2000). The lack of a clear pattern somatic condition variations in *L. sclateri* populations could be due to the fact that localities where best riparian cover exists suffer a very flow fluctuation, whereas in sampling sites with more stable flows the riparian cover is poorer and there is a lower water quality due to the intense agricultural land use.

Hydrologic alteration has been recognized as an important threat to ecological sustainability of rivers and their associated floodplain wetlands (Richter *et al.*, 1996). In this study, different hydrological scenarios related to flow regimes and the presence of artificial-flow habitats derived from anthropogenic impoundment have had an effect on population size structure of target species. However, further studies are needed in order to assess the specific sensitivity of each of those species. Freshwater fish populations or assemblages seem to be sensitive indicators of flow alteration, showing changes in their biological traits and even modifying their life history strategies when flow parameters are altered (Murchie *et al.*, 2008; Mims & Olden, 2013; Vasconcelos *et a*l., 2014). A further knowledge about how size-related variables reflect changes in life-history strategies (as reproduction, recruitment and mortality rate) driven by regulated flow pattern could be an important factor for environmental management. To know the size structure of fish populations expected in natural flow conditions could be used in management programs as a reference point about species and river welfare. Moreover, the application of size structure indexes could be a good complementary tool in freshwater bioassessments in species-poor systems (Benejam *et al.*, 2015).

Conclusions

The flow regimes typical from Mediterranean basins and also regulation impacts, such as the presence of high flow peaks related to reservoir discharges, were important factors shaping size structure of fish populations and, consequently, fish assemblages. Probably, more limnophilic or suprapelagic invasive fishes like *A. alburnus* and *L. gibbosus*, which are not adapted to overtake the seasonal flash flood in their native areas, have more difficulties to establish populations. However, the homogenization of flow regimes (high base daily flow and absence of low-flow periods), together with presence of artificial-flow habitats consequence of impoundment structures, could favour their establishment cohabiting with rheophilic freshwater fish, if the latter are translocated or native.

Alburnus alburnus was the only species whose size-related variables were not significantly affected by different flow regimes or types of stretches. Higher somatic condition was found in populations located in more lentic sites, while lower somatic condition was observed in fluvial stretches where environmental characteristics are more different to its native requirements. In addition, population structure observed in this study supports the population viability of *A. alburnus* in fluvial stretches far from lentic zones in reservoirs.

Reduction of seasonality in flow patterns and presence of artificial-flow habitats seem to be a decisive factor for the success of *L. gibbosus* populations in the Segura River basin. Water withdrawal structures provided artificial-flow habitats more suitable for this lentic species, showing populations with high size diversity and elevated representation of small sizes.

Free-flow habitats were more suitable for the establishment of the rheophilic species *G. lozanoi,* showing populations with representation of all size classes and predominance of medium and longer individuals. On the other hand, stretches with artificial-flow habitats derived of weir structures seems to be a suitable habitat for the survival of smaller individuals.

Populations of the native species *L. sclateri* showed lower somatic condition in sectors from the upper part of the basin, where environmental stress probably related to dry summers and also to cold winters is higher. However, in regulated sectors where environment was more stable, the species showed better fish condition and numerous populations. Fluvial stretches with artificial-flow habitats showed the highest abundance of small individuals.

In general, this study showed that different flow patterns and presence of artificial-flow habitats derived from anthropogenic impoundment have had an influence on some size-related variables and on population size structure of the target species. The responses in size-related variables were species-specific in accordance with their ecological guilds.

The Mediterranean natural flow regime was unsuitable for species typical of stable environments (lentic habitats), while presence of regulation structures buffers drastic flows and provides potential fish refuges and more stable environments, which allowed the development of all target species. The role of fish as indicators of flow alteration (Petchey & Belgrano, 2010; Emmrich *et al*., 2014) and the fact that many population size structure patterns are shaped by biotic and abiotic interactions (De Leeuw *et al*., 2003; Brucet *et al*., 2010) set out a framework where the relationship between both considerations is possible.

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Chapter 5

Reproductive strategy and energy allocation of *Gobio lozanoi* Doadrio & Madeira, 2004 from an invaded Mediterranean basin

Introduction and objectives

One of the major drivers of native biodiversity lost on a global scale are invasive species (Clavero & García-Berthou, 2005), freshwater biotas being especially vulnerable to new invasive fish (Cucherousset & Olden, 2011; García-Berthou *et al.*, 2015). The high rate of new introductions is a serious threat, particularly in areas with high endemism such as the Mediterranean basins (Leunda, 2010; Hermoso & Clavero, 2011; García-Berthou *et al.*, 2015). Consequently, expanding the knowledge of emerging invasive species is a key factor in the fight to preserve biodiversity.

Reproductive strategies of species are the result of the combination of reproductive traits which have been modulated by specific environmental conditions to be successful in a particular environment (Wootton, 1984). Most habitats are subject to periodic changes and such reproductive traits can show a certain degree of adaptive variations for species to adapt to their surrounding environmental conditions (Wootton, 1984; Munro, 1990; Pankhurst & Munday, 2011; Karjalainen *et al.*, 2016). Invasive species have a series of generalists' biological traits and a wide phenotypic plasticity which allow them to survive and adapt to new habitats (Bøhn *et al.*, 2004; García-Berthou, 2007). The reproductive strategy of invasive species is expected to change during the invasion process showing high relative reproductive investment, early maturation and high numbers of small-sized eggs at early stages of establishment, while lower relative reproductive investment, later maturation, low fecundity and larger eggs are found in later stages of the invasion process (Bøhn *et al.*, 2004). A study about life-history traits of invasive fish in small Mediterranean streams showed that most exotic fish species are late-maturing with high fecundity, a few spawnings per year and a short reproductive span, while exotic Iberian fish (allochthonous species) were characterised by early maturity, low fecundity, multiple spawnings per year and a long reproductive span, these traits being more similar to those of the native species (Vila-Gispert *et al.*, 2005).

In reproductive strategies, nutrient acquisition and energy allocation to reproduction are essential for energy balance in order to meet survival, growth and reproduction demands and aim at developing the most competitive strategy (Gadgil & Bossert, 1970; Stearns, 1992). Regarding nutrients and energy allocation, two big strategies can be defined: a) *capital breeders*, as those species which store the energy required before the reproductive period, and b) *income breeders*, as those which acquire the energy required for reproduction from current feeding (Henderson *et al.*, 1996; Stephens *et al.*, 2009; Kennedy *et al.*, 2010; McBride *et al.*, 2015). Total spawners, being semelparous or iteroparous fish, or species with synchronous oocyte development are likely to obtain the required energy before the reproductive event (i.e. *capital breeders*), while many small, batch-spawning fishes with asynchronous oocyte development are expected to be more dependent on current intake of energy from feeding (i.e. *income breeders*) (McBride *et al.*, 2015). However, species can develop a range of intermediate strategies, aiming for the most adequate reproductive strategy for each species and environment (Houston *et al.*, 2007; Ganias, 2013; Wuenschel *et al.*, 2013).

Fish health depends on environmental conditions and food availability and affects reproduction processes. Fish health indexes, such as somatic or hepatosomatic conditions, can be used as an indirect way to assess energy allocation dynamics (Collins & Anderson, 1995; Lambert & Dutil, 1997). Moreover, somatic and hepatosomatic conditions have been associated with oocyte recruitment in several species (Marshall *et al.*, 1999; Rinchard & Kestemont, 2003; McBride *et al.*, 2015).

The Pyrenean gudgeon, *Gobio lozanoi* Doadrio & Madeira 2004 (Actinopterygii, Cyprinidae), is a widely distributed freshwater fish around the Iberian Peninsula whose native role remains unclear (Doadrio *et al.*, 2011; Amat-Trigo, 2017). Some authors consider this species has a high capacity to spread and can behave as dangerous invaders, rapidly increasing their density and occupying new habitats (Elvira, 2010; Doadrio *et al.* 2011), and it has already been suggested that this allochthonous species may have potential impacts on the environment and native species (Leunda, 2010; Amat-Trigo, 2017; Oliva-Paterna *et al.*, 2017). For instance, in the Segura River Basin, *G. lozanoi* can be found from the upper part of the basin to an area near the river mouth (Andreu-Soler *et al.*, 2006), where it has colonized almost all types of water bodies (Torralva *et al.*, 2005; Amat-Trigo *et al.*, 2013; Oliva-Paterna *et al.*, 2014). Moreover, the lack of biological and ecological information on this allochthonous species may be hiding negative effects this species may have on native fish (see Chapter 2 for more information). In spite of this lack of knowledge, there is a scarcity of studies dealing with their biology and reproductive traits (Lobón-Cerviá & Torres, 1984; Lobón-Cerviá *et al.*, 1991; Miñano *et al.*, 2003), and no studies include physiological approach to energy allocation dynamics in reproductive strategies.

From a conservation perspective, there is a clear need for biological information on *G. lozanoi* population traits across the Iberian Peninsula and especially in its non-native river basins which could be an important gap in knowledge that currently hampers effective conservation and management of native species *sensu* Ribeiro & Leunda (2012). As translocated fish, *G. lozanoi* has colonized the Iberian Peninsula, showing certain variability in biological traits (see Chapter 2 for more information). The hypothesis to test was the fact that *G. lozanoi* would show reproductive investment typical of allochthonous species *sensu* Vila-Gispert *et al.* (2005) and an energetic reproduction strategy similar to *income breeding* strategy. Reproductive investment can be understood as the result of the energy balance between survival, growth and reproduction demands in order to aim at developing the most competitive strategy (Stearns, 1992), which also is a relevant factor in reproductive strategies of species (Mcbride *et al.*, 2015). The main goal of the present study was to analyse the reproductive traits and the energy balance strategy, mainly during the reproduction phase, of *G. lozanoi* in an invaded Mediterranean basin in the southeast of the Iberian Peninsula (Segura River basin). The specific objectives of the present work were:

a) To describe the reproductive and fish condition cycles of the non-native *Gobio lozanoi* in the Segura River Basin.
b) To describe the energy allocation (proteins and lipids contents) among tissues in the target fish.
c) To analyse the relationships among reproductive traits, fish condition and patterns of energy allocation.

Materials and methods

Study Area

This study was conducted in the upper and middle parts of the Segura River basin (drainage area of 18,870 km^2) (Fig. 5.1). It is located in the southeast of the Iberian Peninsula and characterised by Mediterranean climate showing a strong climatic and altitudinal gradient and a pronounced spatial and seasonal hydrological variability (Grindlay *et al.*, 2011) (see Chapter 2 for more information).

For the present study, five sampling sites were established along the longitudinal gradient of the basin (Fig. 5.1 and Table 5.1) and located in fluvial sectors with different hydrological regimes. Each sampling site was characterised by the following 8 environmental variables: altitude (Alt) (meters above sea level), ecological status (Status) *sensu* EU Water Framework Directive (Status) (with the following categories: 1 = high; 2 = good; 3 = moderate; 4 = poor), conductivity (µS cm−1), Fluvial Habitat Index (IHF), Riparian Quality Index (RQI), 2015 mean monthly temperature (ºC), mean daily flow (Mdf, m^{3}s^{-1}) and flow variability (Fv, $Q_{90\%}$-$Q_{10\%}$) (Table 5.1). The last two variables were calculated from a time series data of the 1994-2015 hydrological cycles obtained from the official monitoring service of the administrative agency of the Segura River basin.

Table 5.1. Habitat variability values of each sampling site where *Gobio lozanoi* populations were assessed. Altitude (m.a.s.l.); Ecological status (Status) (1-4); Water conductivity (Cond., ±0.1 µs cm^{-1}) (mean and ± 95% CI); Fluvial Habitat Index (IHF); Riparian Quality Index (RQI); Water temperature (Temp., ºC) (mean and ± 95% CI); Mean daily flow (Mdf, m^3 s^{-1}); and Flow variability (Fv, $Q_{90\%}$-$Q_{10\%}$).

Sampling site	Code	Altitude (m.a.s.l.)	Status	Cond. (µs cm^{-1})	IHF	RQI	Temp. (ºC)	Mdf (m^3 s^{-1})	Fv
Tus	TUS	809	1	399.44 ± 9.89	84	65	15.63 ± 2.77	1.88	-2.527
Camping	SE1	685	1	374.33 ± 8.06	61	98	14.71 ± 2.15	3.414	-3.042
Letur	SE2	460	1	362.62 ± 4.21	68	80	13.04 ± 1.59	4.923	-2.167
Bajo Cenajo	SE3	363	2	383.28 ± 3.73	67	77	12.49 ± 1.28	2.567	-8.052
Hoya García	SE4	200	2	686.19 ± 55.20	66	70	16.25 ± 2.01	15.434	-1.443

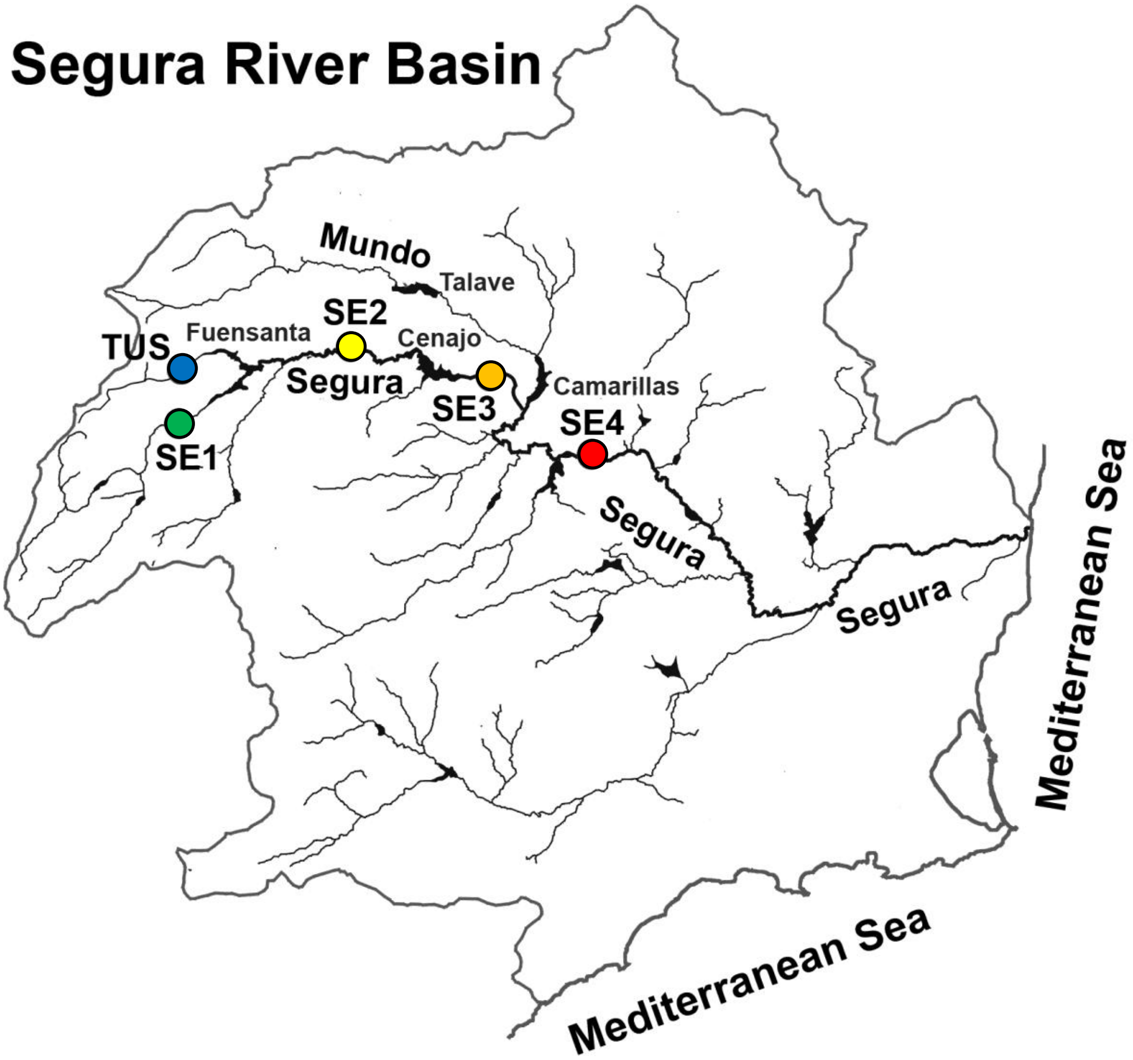

Figure 5.1. Sampling sites location in the Segura River basin. Codes of sampling sites according to the Table 5.1.

Field sampling and laboratory procedures

Electrofishing was used to collect fish during a one-year study period, from January to December 2015. Samples were taken once every two weeks in spring and summer, and monthly during the rest of the year. In order to obtain representative samples from each site relative to the variability of meso and microhabitats, the minimum wadeable sections covered at each sampling site were approximately 100 metres long. A total of 2333 *G. lozanoi* were caught (TUS: 437; SE1: 478; SE2: 385; SE3: 485 and SE4: 548) and, in accordance with Spanish regulations, immediately sacrificed in a water tank with an overdose of anaesthetic solution and placed on ice until stored at -20°C in the laboratory.

Fork length (L_F ± 1 mm), total and eviscerated masses (M_T and M_E ± 0.1g) and organ masses (hepatic and gonad, M_H and M_G ± 0.001 g) of a subsample of 1982 fish were recorded (TUS: 382; SE1: 403; SE2: 365; SE3: 366 and SE4: 466). Gonads were visually inspected for sex determination (male, female or immature). A subsample of 133 mature specimens (110 females and 23 males), with fork length ranging from 7.2 to 11.2 cm was used to estimate fecundity, oocyte size and physiological macronutrients (protein and lipid content in tissues). Due to protein and lipid quantification methods, there were not enough testis masses to perform physiological analysis at each sampling site. To quantify protein and lipid content quantification in the muscle, liver and gonad, samples were weighted and protein levels were determined using the method of Bradford (1976) and expressed as percentages. Total lipids were extracted following the method of Folch *et al.* (1957). Samples were weighted and homogenized in 5 ml of chloroform/methanol (2:1 v/v), and washed with KCl (0.88% w/v). The weight of lipids was determined gravimetrically after evaporation of the solvent and expressed as percentages. These fish were aged counting true annuli from scales taken between lateral line and dorsal fin origin.

Ovarian development and fecundity were studied using the gravimetric method (Bagenal & Braum, 1978). To make sure that the ovary was homogenous in structure (number and size of oocytes), small portions of anterior, middle and distal parts were compared and no significant differences were found (ANOVA, $P > 0.05$). Therefore, all oocytes present in a subsample from the mid-region of the right ovarian lobe (5% of the total weight of the gonad) were placed in Gilson liquid, shaken periodically to soften gonadal tissue and to disperse oocytes, washed with distilled water and preserved in 70% ethanol for following analyses (Miñano *et al.*, 2003). The total number and size of oocytes in each gonad subsample were measured using the image processing program ImageJ v1.80 (available at https://imagej.nih.gov/ij/). Fecundity was determined in 39 mature females caught from April to July. Fecundity was analysed at three levels: potential (Fec_{POT}), absolute (Fec_{ABS}) and batch fecundity (Fec_{BAT}). These levels were determined by counting the total number of opaque and vitellogenic oocytes, total number of vitellogenic oocytes and total number of vitellogenic oocytes of the last mode representing size before spawning, respectively (Bagenal, 1978; Murua *et al.*, 2003). Oocyte diameter at each level of fecundity ($Ø_{POT}$, $Ø_{ABS}$, $Ø_{BAT}$) and maximum diameter ($Ø_{MAX}$) were assessed.

Statistical analyses

Sex-ratio was analysed for the whole population. The degree of significance of the obtained results was established in $\chi 2$ at a *p*-value of $P < 0.05$. Linear regressions of fecundity to fork length were fitted by least-squares method to log-transformed data.

Analyses of length-mass relationships were performed to study temporal variation in fish conditions (somatic and hepatosomatic condition) and gonadal activity. The statistical approach included the application of a covariance analysis (ANCOVA) using M_E, M_H and M_G as dependent variables, L_F as the covariate (log-transformed data) and reproductive stage as a factor. The analysis was developed by sampling site and sex separately. Differences between dependent–covariate relationships were tested to check that the covariate by-factor interaction (homogeneity of slopes) was significant ($P <$ 0.05). If the covariate by-factor interaction was not significant, standard ANCOVA was applied to obtain predicted values (predicted M_E, M_H and M_G values). When differences were found, a post hoc Bonferroni test for multiple comparisons was performed. Student's t-test was used to evaluate differences in fish conditions somatic and hepatosomatic condition), gonadal activity, and protein and lipid content between sexes. Analyses of variance (ANOVA) were performed to determine differences in protein and lipid content among the different temporal phases, followed by the Tukey HSD test post-hoc comparisons if significant differences among populations were found. When data did not show homogeneity of variances, Welch's analysis of variance (ANOVA) followed by T3 of Dunnett for pairwise multiple comparisons were developed. Non parametric tests of Kruskal–Wallis H-test and Mann–Whitney U-test were used when data did not fit normal distribution.Relations between fish condition, gonadal activity, fecundity, oocyte diameter and percentage of proteins and lipids by tissue were analysed using Spearman's correlation coefficients.

Size of first maturity was estimated after running binary logistic regressions (immature-mature individuals) for each sampling site by sex. In this study, only to determine maturity of individuals gonadosomatic index (GSI), calculated as GSI = MG / ME * 100 (*sensu* Bagenal, 1978), was used. Individuals were considered mature when this index was higher than 8.5 in females or 1.7 in males in spring and summer. To establish these thresholds, GSI values of individuals whose gonads were completely inactive (November to February) were plotted and the value of the third quartile of data distribution was selected.

Statistical analyses were performed with the SPSS® software package v. 15.0 and a significance level of 0.05 was accepted.

Results

Reproductive cycle and temporal variation in fish condition

The results of the ANCOVAs' test to estimate the effects of the factor on the L_{F}-M_{E}, L_{F}-M_{H} and L_{F}-M_{G} relationships are shown in Tables 5.2 and 5.3. In both sexes, significant changes were observed among reproductive stages in the five sampling sites for fish somatic condition (predicted M_{E} values), hepatosomatic condition (predicted M_{H} values) and gonadal activity (predicted M_{G} values) (Table 5.2 and 5.3).

The reproductive cycle was fitted by the ANCOVA predicted M_{G} values as a Gonadosomatic index (GSI) showing significant temporal changes in the gonadal activity (Fig. 5.2).

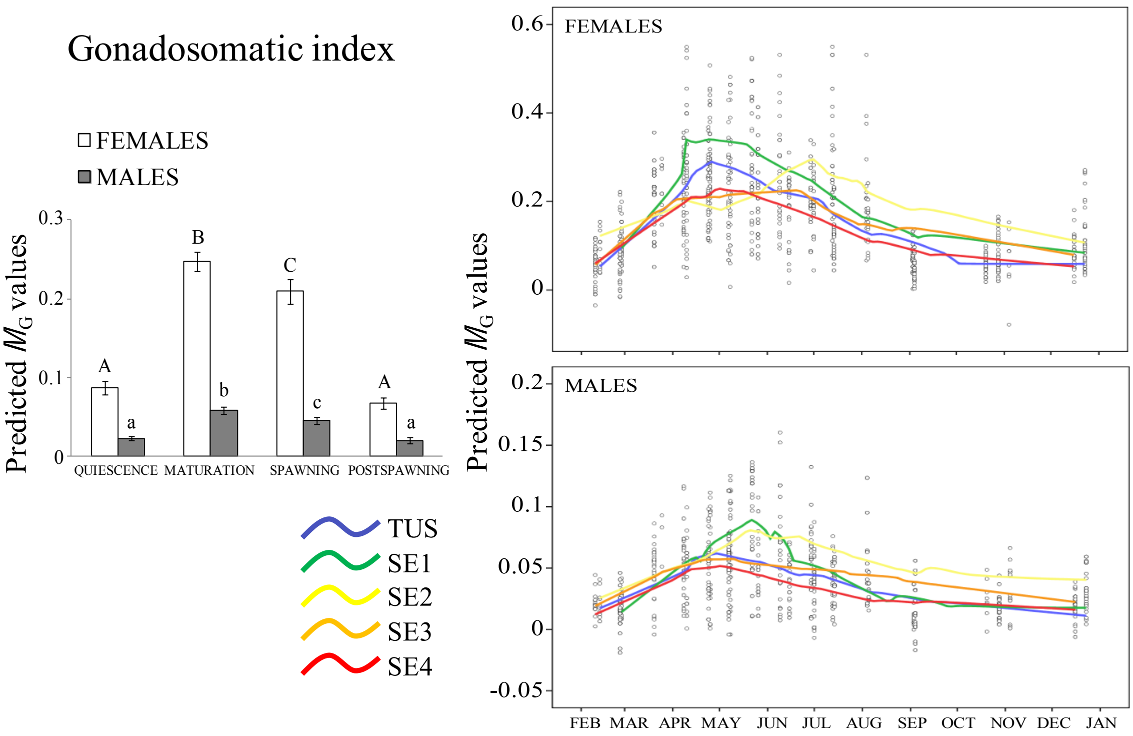

Figure 5.2. On the left, mean predicted M_{G} values by ANCOVA (L_{F} as covariate) in each reproductive stage (quiescence, maturation, spawning and postspawning) for both sexes of *Gobio lozanoi*. Significant differences (Kruskal–Wallis H-test, $P < 0.05$) among reproductive stages in female (capital letters) and in males (lowercase letters) are presented. On the right, temporal variation in gonad activity (predicted M_{G} values) along the study period for the five studied populations (TUS, SE1, SE2, SE3 and SE4). The lines represent the adjusted model *Loess* for each population.

Table 5.2. Results of ANCOVA analyses of the (a) L_F-M_E, (b) L_F-M_H and (c) L_F-M_G relationships in MALES of *Gobio lozanoi* at each sampling site. Fork length (L_F) is the covariate and reproductive stage is the fixed factor. M_E: eviscerated mass; M_H: hepatosomatic mass; M_G: gonadal mass. Degrees of freedom, *F*-statistics and *P* values (significant effects in bold) are presented.

Samplig site	TUS			SE1			SE2			SE3			SE4		
MALES	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
(a) Relation L_F - M_E															
Preliminary ANCOVA															
Length	1	1565.03	< **0.001**	1	1345.77	< **0.001**	1	1731.17	< **0.001**	1	4427.82	< **0.001**	1	2415.10	< **0.001**
Reproductive stage	3	1.72	0.172	3	0.028	0.994	3	0.868	0.461	3	3.75	**0.012**	3	1.03	0.382
Length x Reproductive stage	1	1.49	0.224	3	0.045	0.987	3	0.841	0.476	3	4.71	**0.004**	3	1.40	0.246
Standard ANCOVA															
Length	1	2309.66	< **0.001**	1	2715.72	< **0.001**	1	2273.40	< **0.001**				1	5538.39	< **0.001**
Reproductive stage	3	11.34	< **0.001**	3	15.43	< **0.001**	3	0.163	0.921				3	6.94	< **0.001**
(b) Relation L_F - M_H															
Preliminary ANCOVA															
Length	1	137.07	< **0.001**	1	85.39	< **0.001**	1	161.84	< **0.001**	1	207.46	< **0.001**	1	103.23	< **0.001**
Reproductive stage	3	0.529	0.664	3	3.43	**0.020**	3	3.04	**0.034**	3	1.49	0.218	3	0.92	0.435
Length x Reproductive stage	3	0.474	0.702	3	3.89	**0.011**	3	3.339	**0.024**	3	2.09	0.104	3	1.02	0.384
Standard ANCOVA															
Length	1	229.22	< **0.001**							1	202.78	< **0.001**	1	243.858	< **0.001**
Reproductive stage	3	1.68	0.179							3	4.67	**0.004**	3	5.00	**0.002**
(c) Relation L_F - M_G															
Preliminary ANCOVA															
Length	1	90.50	< **0.001**	1	97.45	< **0.001**	1	125.95	< **0.001**	1	141.64	< **0.001**	1	95.02	< **0.001**
Reproductive Stage	3	14.847	< **0.001**	3	11.30	< **0.001**	3	9.77	< **0.001**	3	2.48	0.063	3	3.40	< **0.001**
Length x Reproductive stage	3	17.19	< **0.001**	3	14.34	< **0.001**	3	12.19	< **0.001**	3	3.74	**0.012**	3	16.75	< **0.001**
Standard ANCOVA															
Length										1	145.36	< **0.001**			
Reproductive stage										3	30.26	< **0.001**			

Table 5.3. Results of ANCOVA analyses of the (a) L_F-M_E, (b) L_F-M_H and (c) L_F-M_G relationships in FEMALES of *Gobio lozanoi* at each sampling. Fork length (L_F) is the covariate and reproductive stage is the fixed factor. M_E: eviscerated mass; M_H: hepatosomatic mass; M_G: gonadal mass. Degrees of freedom, F-statistics and P values (significant effects in bold) are presented.

Sampling site		**TUS**			**SE1**			**SE2**			**SE3**			**SE4**	
FEMALES	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
(a) Relation L_F - M_E															
Preliminary ANCOVA															
Length	1	647.36	**< 0.001**	1	1803.07	**< 0.001**	1	2785.77	**< 0.001**	1	2221.93	**< 0.001**	1	4840.60	**< 0.001**
Reproductive stage	3	2.22	0.092	3	0.415	0.743	3	0.954	0.417	3	7.23	**< 0.001**	3	1.57	0.197
Length x Reproductive stage	3	2.43	0.072	3	0.714	0.545	3	0.861	0.463	3	7.07	**< 0.001**	3	1.52	0.210
Standard ANCOVA															
Length	1	883.97	**< 0.001**	1	2185.23	**< 0.001**	1	2863.25	**< 0.001**				1	6002.29	**< 0.001**
Reproductive stage	3	5.08	**0.003**	3	22.38	**< 0.001**	3	1.56	0.201				3	5.92	**0.001**
(b) Relation L_F - M_H															
Preliminary ANCOVA															
Length	1	78.442	**< 0.001**	1	63.73	**< 0.001**	1	433.84	**< 0.001**	1	133.77	**< 0.001**	1	346.66	**< 0.001**
Reproductive stage	3	6.37	**0.001**	3	2.28	0.082	3	10.88	**< 0.001**	3	2.84	**0.041**	3	4.59	**0.004**
Length x Reproductive stage	3	7.17	**< 0.001**	3	2.98	**0.034**	3	13.04	**< 0.001**	3	3.27	**0.023**	3	5.57	**0.001**
Standard ANCOVA															
Length				1	92.59	**< 0.001**									
Reproductive stage				3	24.47	**< 0.001**									
(c) Relation L_F - M_G															
Preliminary ANCOVA															
Length	1	29.23	**< 0.001**	1	109.93	**< 0.001**	1	236.72	**< 0.001**	1	43.89	**< 0.001**	1	141.01	**< 0.001**
Reproductive stage	3	3.78	**0.014**	3	6.15	**0.001**	3	10.34	**< 0.001**	3	1.767	0.157	3	6.19	**< 0.001**
Length x Reproductive stage	3	4.77	**0.004**	3	9.35	**< 0.001**	3	14.67	**< 0.001**	3	3.16	**0.027**	3	10.04	**< 0.001**
Standard ANCOVA															
Length										1	91.58	**< 0.001**			
Reproductive stage										3	35.83	**< 0.001**			

Both sexes showed a similar reproductive cycle in which four stages were identified based on the GSI values (predicted M_G values) of the gonadal activity (Fig. 5.2): (1) the quiescence stage, with low values of GSI in winter; (2) the maturation stage, when GSI values rise up steeply especially in March and reach the maximum values at the beginning of May in females (except females from SE2) and also in males from TUS, SE3 and SE4, but in the case of males from SE1 and SE2 maturation is observed until late May; (3) the spawning stage, when GSI keeps the values or decreases moderately until late summer or early fall, and (4) the regression stage or postspawning, when GSI continues to decrease and reaches minimum values (Fig. 5.2). Moreover, there are certain differences in the reproductive cycle in sampling sites that are much clearer in females which will be analysed in depth in Chapter 6 of this PhD thesis. The female gonadosomatic index was significantly higher than the male's in all reproductive stages (Student's t-test, quiescence stage: $t = -14.559$ $P < 0.001$; maturation stage: $t = -28.968$ $P < 0.001$; spawning stage: $t = -20.210$ $P < 0.001$; postspawning stage: $t = -11.536$ $P < 0.001$).

Somatic condition (SC) and hepatosomatic condition (HS) were found to vary over the reproductive cycle (Fig. 5.3). Somatic condition showed significant differences among reproduction stages in all sampling sites in both sexes when sampling sites are analysed individually, with the exception of SE2 (male ANCOVA, $F_{(1,\ 3,\ 79)} = 0.163$, $P = 0.921$; female ANCOVA, $F_{(1,\ 3,\ 141)} = 1.561$, $P = 0.201$) (Table 5.2 and 5.3). SC increased from the quiescence stage to the reproductive event and then, kept on decreasing until the end of the spawning stage in TUS and SE1populations in males and females. However, in SE2, SE3 and SE4 populations, somatic condition was more variable, showing higher values at the end of the spawning stage (Fig. 5.3). The hepatosomatic condition of males from TUS, did not present differences in reproductive stages (ANCOVA, $F_{(1,\ 3,\ 71)} = 1.680$, $P = 0.179$), while significant differences were found in both sexes in the other sampling sites (Table 5.2 and 5.3). HC cycle in males showed high values at the beginning of the reproductive cycle and decreased from the quiescence stage reaching the lowest values at spawning. However, the female HC cycle showed different patterns at sampling site level (Fig. 5.3). Male somatic condition was significantly higher than female's in most reproductive stages (Student's t-test, quiescence stage: $t = 3.831$, $P < 0.001$; maturation stage: $t = 4.006$, $P < 0.001$; spawning stage: $t = 4.295$, $P < 0.001$), whereas female hepatosomatic condition showed to be higher than males' during maturation and at spawning (Student's t-test, maturation stage: $t = -7.130$, $P < 0.001$; spawning stage: $t = -5.498$, $P < 0.001$).

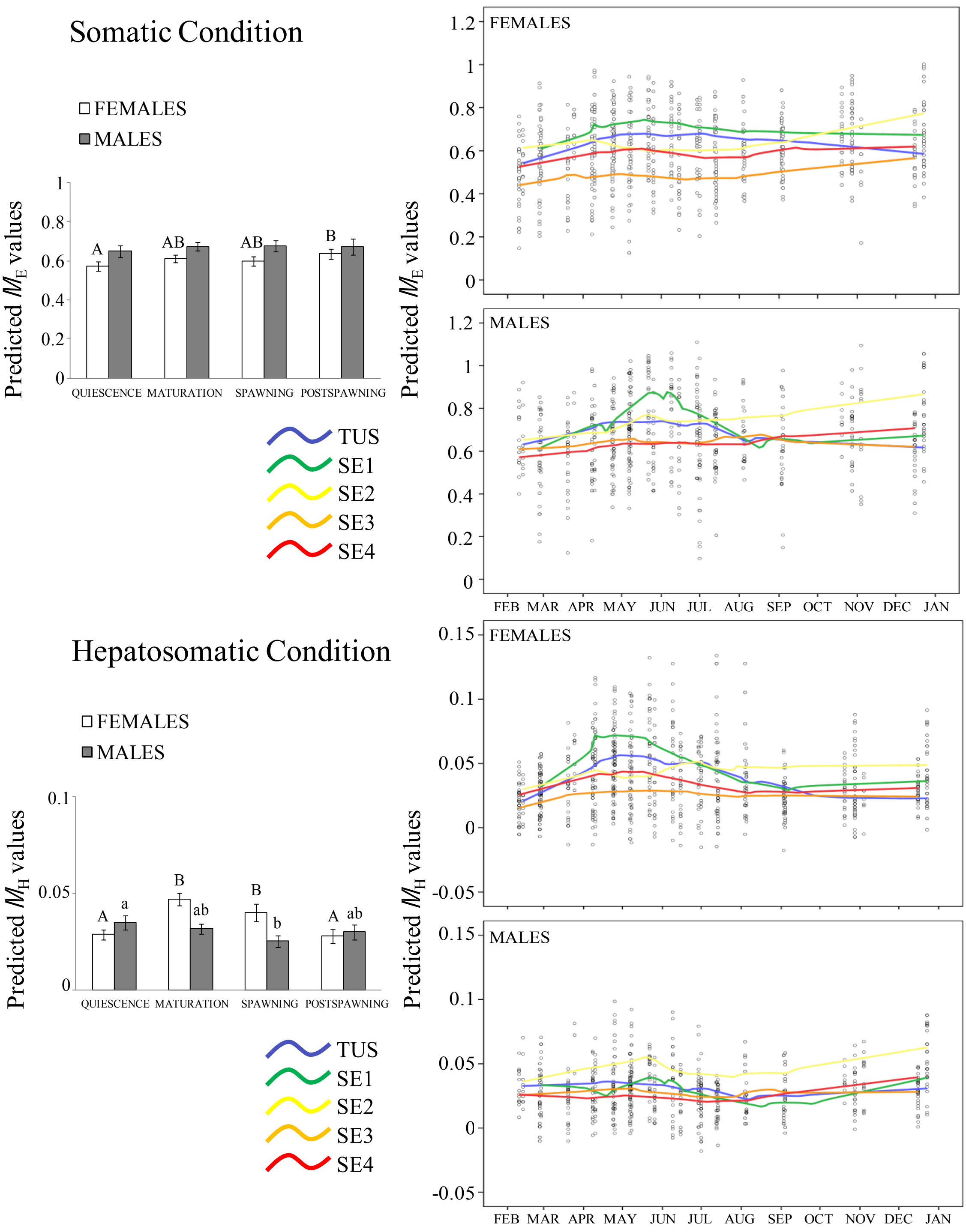

Figure 5.3. On the left, mean predicted M_E and M_H values by ANCOVA (fork length as covariate) in each reproductive stage for both sexes of *Gobio lozanoi*. Letters show significant differences (ANOVA and Kruskal–Wallis H-test, $P < 0.05$ and Tukey post hoc tests) among reproductive stages in female (capital letters) and in males (lowercase letters) data. On the right, temporal variation in gonad activity (predicted M_E and M_H values) along the study period for the five studied (TUS, SE1, SE2, SE3, and SE4). The lines represent the adjusted model *Loess* for each population.

Reproduction traits

The overall sex-ratio (696 males, 928 females) was significantly skewed towards females (χ^2= 33.14, $P < 0.001$) in the whole study period, with females being significantly more abundant in all sampling sites with the exception of SE3, which did not show differences between males and females.

Length at first maturity in males ranged between 3.55 cm L_F in SE3 and 6.26 cm L_F in SE2, while female range was between 4.28 cm L_F in SE3 and 6.60 cm L_F in TUS. Above these lengths all individuals were considered mature (Fig. 5.4).

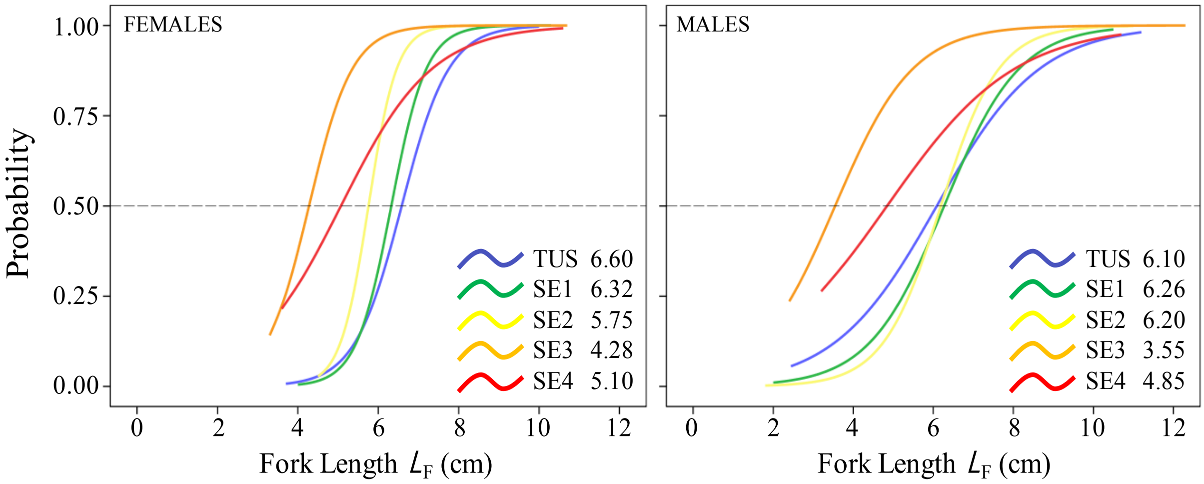

Figure 5.4. Predicted probability of maturity according to fork length for females and males in the five populations of *Gobio lozanoi* studied in the Segura river basin.

Oocytes longer than 0.25 mm of diameter were considered opaque and all oocytes above 0.55 mm of diameter were vitellogenic. Fecundity and oocyte diameter data from each sampling site is summarized in Tables 5.4 and 5.5, respectively.

No significant differences were found in fecundity and oocyte diameters by age (ANCOVA, $P > 0.05$). Fork length was not significant as a covariable when oocyte diameters were analysed, which indicates no effect of fish size on egg diameters in the studied fish.

Table 2.4. Mean, minimum and maximum values of potential fecundity (Fec$_{POT}$), absolute fecundity (Fec$_{ABS}$) and batch fecundity (Fec$_{BAT}$). Linear regression of fecundities and fork length were shown. Variable coefficients are given and significant *P*-values in bold.

Sampling site	Potential fecundity			Absolute fecundity			Batch fecundity		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
TUS	239.3	89	401	152.4	71	250	65.8	37	101
	LogFec$_{POT}$ = -3.64+ 6.30LogL_F			LogFec$_{VIT}$ = -1.61 + 3.99 LogL_F			LogFec$_{BAT}$= -1.94 + 3.99 LogL_F		
	r^2=0.82		**P=0.001**	r^2=0.54		**P=0.015**	r^2=0.72		**P=0.004**
SE1	311.25	111	461	194.58	78	324	79	33	152
	LogFec$_{POT}$ = -4.34+ 7.05 LogL_F			LogFec$_{VIT}$ = -3.78+ 6.27 LogL_F			LogFec$_{BAT}$ = -4.51 + 6.63 LogL_F		
	r^2=0.62		**P=0.007**	r^2=0.56		**P=0.013**	r^2=0.70		**P=0.003**
SE2	248.13	136	389	168.5	83	317	85	42	140
	LogFec$_{POT}$ = -2.02 + 4.63 LogL_F			LogFec$_{VIT}$ = -3.48 + 5.97 LogL_F			LogFec$_{BAT}$ = -3.44 + 5.58 LogL_F		
	r^2=0.75		**P=0.012**	r^2=0.77		**P=0.010**	r^2=0.63		**P=0.018**
SE3	117.4	41	181	79	36	103	56	35	74
	LogFec$_{POT}$ = -3.09 + 5.51 LogL_F			LogFec$_{VIT}$ = -0.86 + 1.15 LogL_F			LogFec$_{BAT}$ = -4.14 + 6.17 LogL_F		
	r^2=0.94		**P=0.033**	r^2=0.34		P=0.417	r^2=0.86		P=0.073
SE4	220.25	70	427	132.5	41	252	85.5	37	124
	LogFec$_{POT}$ = -3.67 + 6.12 LogL_F			LogFec$_{VIT}$ = -3.94 + 6.17 LogL_F			LogFec$_{BAT}$ =-2.11 + 4.12 LogL_F		
	r^2=0.91		**P=0.044**	r^2=0.95		**P=0.023**	r^2=0.77		P=0.121

Table 5.5. Mean, minimum and maximum values of oocyte diameter (mm) of the following oocyte groups: (1) opaque and vitellogenic oocytes; (2) vitellogenic oocytes; and (3) vitellogenic oocytes of the last mode representing size before spawning.

Sampling site	Potential Fecundity			Absolute Fecundity			Batch Fecundity		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
TUS	0.728	0.615	0.891	0.904	0.790	1.021	1.169	1.087	1.274
SE1	0.742	0.620	0.951	0.920	0.805	1.052	1.232	1.057	1.442
SE2	0.712	0.658	0.855	0.877	0.803	0.948	1.035	0.906	1.174
SE3	0.731	0.570	0.871	0.838	0.733	0.995	0.904	0.795	1.161
SE4	0.705	0.676	0.735	0.896	0.851	0.925	0.993	0.940	1.065

Protein and lipid contents

Percentages of proteins and lipids were analysed in gonads (ovaries and testis), muscles and liver. Significant differences in percentages of proteins and lipids were found in tissues during the whole period studied (Table 5.6). Females showed the maximum protein values in gonads and maximum lipid values in liver, while males presented the highest protein and lipid values in the liver (Table 5.6). After comparing percentages of macronutrients in tissues by sex, results revealed that females showed significant higher protein and lipid percentages than males in muscles (Student's t-test, t= -4.463, $P <$

0.001; t= -3.971, $P < 0.001$, respectively), while percentage of protein in the liver was higher in males (Student's t-test, t= 4.514, $P < 0.001$). In gonads, ovary protein content was higher (Student's t-test, t= -9.746, $P < 0.001$), but testis showed higher values of lipid content (Student's t-test, t= 3.141, $P = 0.005$).

Table 5.6. Mean, minimum and maximum percentage values of lipids and proteins in each tissue of *Gobio lozanoi*. Number of individuals *n*, and Kruskal-Wallis test.

			Muscle %			Liver %			Gonad %		Kruskal-Wallis	
		n	Range	Mean	*n*	Range	Mean	*n*	Range	Mean	χ^2	*P*
Female	Protein	110	4.18-11.10	6.95	108	4.10-15.14	9.69	107	0.28-25.59	9.99	86.016	< 0.001
	Lipid	108	0.40-1.35	0.73	105	0.52-18.79	6.94	109	1.05-9.09	3.00	265.237	< 0.001
Male	Protein	23	3.49-8.18	5.67	23	9.26-18.13	12.51	23	0.16-8.99	2.68	54.916	< 0.001
	Lipid	23	0.39-0.97	0.58	23	2.45-22.42	7.18	21	1.84-8.73	4.65	46.484	< 0.001

Percentages of protein and lipid content in tissues showed significant differences during the reproductive cycle (Fig. 5.5). Moreover, lipid-protein dynamic was different between sexes (Fig. 5.5). Lipid percentage in muscle decreases from quiescence to spawning stages in both sexes (ANOVA, female $F_{(3,106)} = 11.035$, $P < 0.001$; male $F_{(3,19)} = 6.330$, $P = 0.008$) (Fig. 5.5). In liver, protein percentages in females reached higher values at spawning (Welch ANOVA, $F_{(3,42.438)} = 3.598$, $P = 0.021$), but no differences were found in the percentages of lipid contents in the liver (Fig. 5.5). No significant differences were found in percentages of protein and lipid contents in the liver in males during the reproductive cycle. Protein percentage in ovary increased until spawning stage (ANOVA, $F_{(3,103)} = 8.731$, $P < 0.001$), but decreased in testis (ANOVA, $F_{(2,19)} = 9.670$, $P = 0.001$) (Fig. 5.5). No significant differences were found in gonadal lipids during the reproductive cycle (Fig. 5.5).

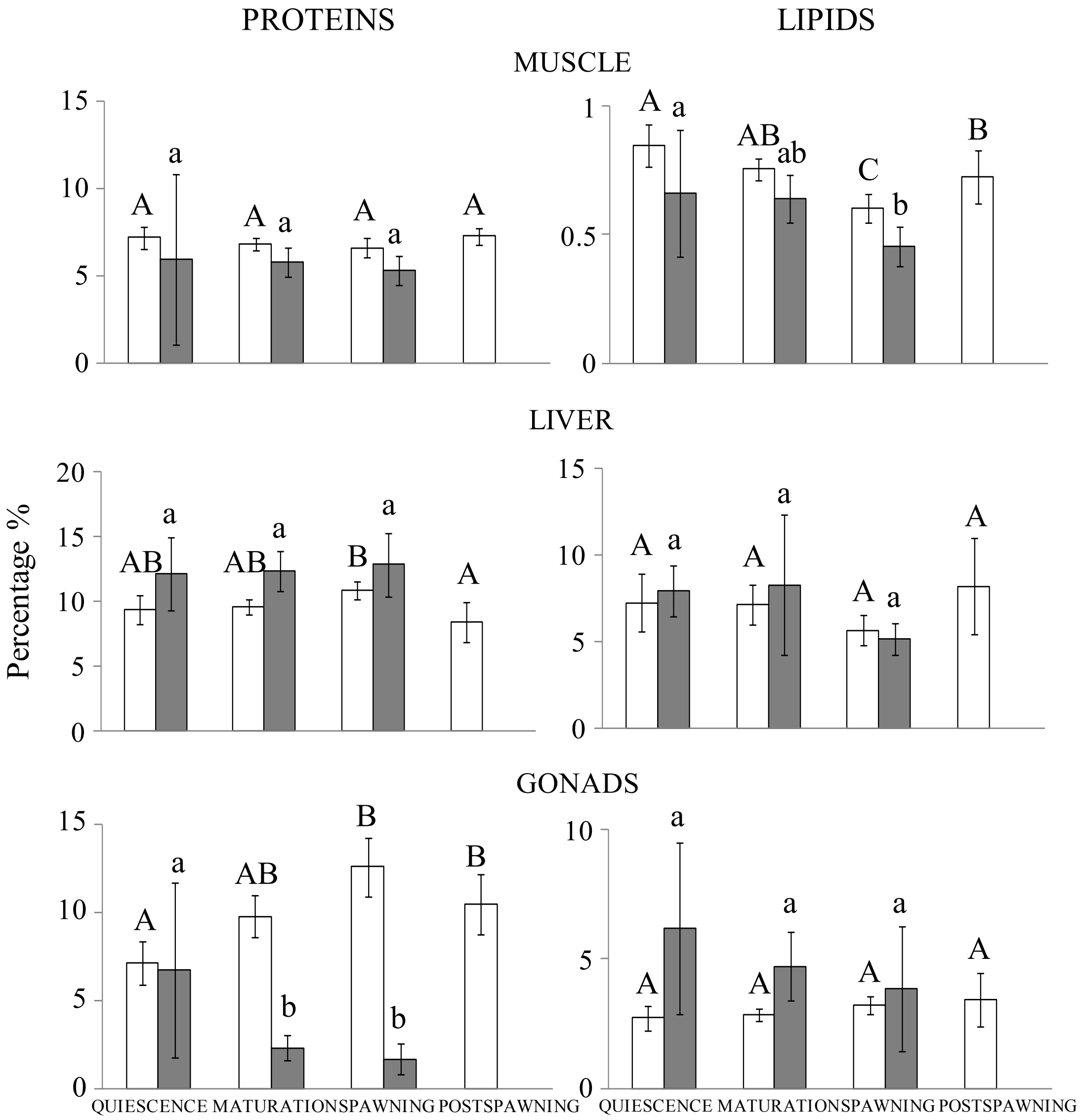

Figure 5.5. Mean and ± 95% CI percentages of proteins and lipids in muscle, liver and gonads by reproductive stages. White bars represent Female values and grey bars Male ones. The letters show significant differences (Welch ANOVA and ANOVA, $P < 0.05$) among reproductive stages by post hoc comparison Tukey test. Capital letters showed female data and lower case letters male data.

Fish metrics relationships

Tables 5.7 and 5.8 show the correlation matrix of the following fish variables: fish condition (somatic SC and hepatosomatic HC), gonad activity (GSI), fecundity, oocyte diameter and percentage of proteins and lipids in each tissue.

Fish conditions and GSI were positively correlated with fecundity and percentage of ovary proteins (Table 5.8). Somatic condition had a positive relationship with batch oocyte diameter, and hepatosomatic condition and GSI were positively correlated with potential, absolute and maximum oocyte diameters. Fish conditions and GSI in females were positively correlated with percentage of ovary protein, whereas SC and HC had a negative relationship with percentage of muscle lipids (Table 5.8). Fish conditions showed a positive relation with percentages of muscle proteins in males (Table 5.7).

In females, percentages of ovary proteins and lipids were positively correlated with absolute fecundity and oocyte diameters ($Ø_{POT}$, $Ø_{ABS}$, $Ø_{BAT}$, $Ø_{MAX}$). Egg size of absolute fecundity ($Ø_{ABS}$) was positively related to the percentage of proteins in liver and negatively related to the percentage of muscle lipids (Table 5.8). Percentages of proteins and lipids also had an influence on other tissues (Table 5.7 and Table 5.8).

Table 5.7. Correlation matrix of fish somatic condition (SC), hepatosomatic condition (HC), gonad activity (GSI), and proteins (% P_{LIV}, % P_{MUS}, % P_{GON}) and lipids (% L_{LIV}, % L_{MUS}, % L_{GON}) percentages by tissues in MALES. Spearman's coefficient above the diagonal and *P*-values below the diagonal (**significance level $P < 0.01$; * significance level $P < 0.05$).

	SC	HC	IGS	% P_{MUS}	% P_{LIV}	% P_{GON}	% L_{MUS}	% L_{LIV}	% L_{GON}
SC	-	0.845**	0.541**	0.447*	-0.152	-0.109	-0.207	0.018	-0.079
HC	< 0.001	-	0.443*	0.612**	-0.120	0.204	-0.031	0.065	0.053
IGS	0.008	0.034	-	0.269	-0.134	-0.172	0.206	0.034	-0.027
% P_{MUS}	0.032	0.002	0.215	-	-0.046	0.220	0.102	-0.124	-0.025
% P_{LIV}	0.488	0.587	0.541	0.835	-	-0.092	0.056	-0.208	-0.092
% P_{GON}	0.620	0.350	0.434	0.313	0.677	-	0.364	0.266	0.194
% L_{MUS}	0.343	0.888	0.345	0.642	0.799	0.088	-	0.583**	0.583**
% L_{LIV}	0.936	0.767	0.879	0.573	0.340	0.219	0.004	-	0.742**
% L_{GON}	0.735	0.821	0.909	0.915	0.691	0.401	0.006	< 0.001	-

Table 5.8. Correlation matrix of fish somatic condition (SC), hepatosomatic condition (HC), gonad activity (GSI), fecundity (Fec$_{POT}$, Fec$_{ABS}$, Fec$_{BAT}$), oocyte size (Ø$_{POT}$, Ø$_{ABS}$, Ø$_{BAT}$, Ø$_{MAX}$) and proteins (% P$_{LIV}$, % P$_{MUS}$, % P$_{GON}$) and lipids (% L$_{LIV}$, % L$_{MUS}$, % L$_{GON}$) percentages by tissues in FEMALES. Spearman's coefficient above the diagonal and *P*-values below the diagonal (**significance level $P < 0.01$; * significance level $P < 0.05$).

	SC	HC	IGS	Fec$_{POT}$	Fec$_{ABS}$	Fec$_{BAT}$	Ø$_{POT}$	Ø$_{ABS}$	Ø$_{BAT}$	Ø$_{MAX}$	% P$_{MUS}$	% P$_{LIV}$	% P$_{OVA}$	% L$_{MUS}$	% L$_{LIV}$	% L$_{OVA}$
SC	-	0.713**	0.426**	0.491**	0.386**	0.749**	0.032	0.155	0.347*	0.142	-0.054	0.014	0.234*	-0.267**	-0.146	-0.037
HC	<0.001	-	0.816**	0.415**	0.635**	0.755**	0.465**	0.472**	0.186	0.556**	0.016	0.038	0.435**	-0.215*	-0.031	-0.019
IGS	<0.001	<0.001	-	0.267**	0.611**	0.748**	0.570**	0.470**	0.103	0.627**	-0.017	0.041	0.282**	-0.130	-0.101	-0.060
Fec$_{POT}$	<0.001	<0.001	0.005	-	0.695**	0.744**	0.079	0.136	0.401**	0.228*	0.087	-0.259**	0.173	0.041	0.161	0.073
Fec$_{ABS}$	<0.001	<0.001	<0.001	<0.001	-	0.768**	0.732**	0.680**	0.466**	0.780**	0.088	-0.126	0.467**	-0.092	0.167	0.233*
Fec$_{BAT}$	<0.001	<0.001	<0.001	<0.001	<0.001	-	0.015	0.058	0.173	0.212	0.116	-0.308*	-0.054	-0.030	-0.018	-0.038
Ø$_{POT}$	0.744	<0.001	<0.001	0.413	<0.001	0.924	-	0.925**	0.383*	0.874**	-0.029	0.071	0.587**	-0.111	0.055	0.565**
Ø$_{ABS}$	0.138	<0.001	<0.001	0.192	<0.001	0.720	<0.001	-	0.570**	0.922**	-0.005	0.216*	0.682**	-0.326**	0.093	0.661**
Ø$_{BAT}$	0.023	0.246	0.520	0.009	0.002	0.279	0.013	<0.001	-	0.896**	-0.190	0.107	0.384**	-0.234	-0.030	0.284
Ø$_{MAX}$	0.141	<0.001	<0.001	0.016	<0.001	0.184	<0.001	<0.001	<0.001	-	-0.034	0.054	0.605**	-0.174	0.132	0.583**
% P$_{MUS}$	0.581	0.870	0.860	0.368	0.387	0.470	0.760	0.959	0.234	0.724	-	-0.070	-0.054	0.096	0.175	0.134
% P$_{LIV}$	0.888	0.695	0.675	0.006	0.216	0.050	0.460	0.036	0.507	0.574	0.466	-	0.119	-0.255**	-0.279**	0.030
% P$_{OVA}$	0.015	<0.001	0.003	0.072	<0.001	0.739	<0.001	<0.001	0.013	<0.001	0.574	0.217	-	-0.213*	0.143	0.368**
% L$_{MUS}$	0.005	0.025	0.178	0.669	0.365	0.852	0.250	0.001	0.141	0.060	0.317	0.007	0.026	-	0.240*	-0.083
% L$_{LIV}$	0.139	0.754	0.303	0.099	0.104	-0.910	0.576	0.380	0.855	0.179	0.073	0.004	0.146	0.013	-	0.141
% L$_{OVA}$	0.707	0.847	0.533	0.450	0.020	0.813	0.003	<0.001	0.025	0.001	0.162	0.753	<0.001	0.389	0.148	-

Discusion

During this study, similar temporal dynamics of the gonadosomatic index were presented to both sexes. GSI increased since the middle of February, reaching the maximum value in April-May in TUS, SE1 and SE3 populations, and in June-July in SE2 and SE4, to then finish the reproductive period in September for all 5 populations (see Chapter 6 for more information). Miñano *et al.* (2003) found a similar pattern in the GSI for a population of *G. lozanoi* in the upper area of the Segura basin. However, in other non-native populations of *G. lozanoi* located more to the north of the Iberian Peninsula, shorter maturation and spawning periods were observed, starting in March (Jarama River) or April (Ucero and Moro rivers) and ending in August or September (Lobón-Cerviá &Torres, 1984; Lobón-Cerviá *et al.*, 1991). Only a population inhabiting a reservoir finished the cycle later in October (Lobón-Cerviá & Torres, 1984). Changes in environmental parameters such as temperature, which controls maturation and spawning in fish, are less pronounced in reservoirs and these may cause later spawns (Wootton, 1990; Mule-Akash & Sarve-Neha, 2017). Although gonadal activity was simultaneous in males and females, the GSI was systematically higher in females than in males during the whole reproductive cycle. This difference between sexes has also been found in other non-native populations of *G. lozanoi* in the Iberian Peninsula (Lobón-Cerviá & Torres, 1984; Lobón-Cerviá *et al.*, 1991; Miñano *et al.*, 2003).

Fish conditions showed different patterns for females and males, suggesting that condition investment was not the same for both sexes during the reproductive cycle. The somatic condition of females increased from the beginning of the reproductive cycle until the end of postspawning period, while the somatic condition of males was stable during the cycle. Somatic condition increased in the month prior to spawning also in females in the upper area of the Segura basin (Miñano *et al.*, 2003). These patterns were not observed in other non-native populations of *G. lozanoi* in the Iberian Peninsula, which showed two peak values of somatic condition at the beginning and at the end of spawning, minimum values in October (Lobón-Cerviá & Torres, 1984), then somatic condition increased in winter and spring, and was followed by a sudden decrease in summer and until September (Lobón-Cerviá *et al.*, 1991). Somatic condition was higher in males than in females during the reproductive cycle. Such higher somatic condition in males was also found in a previous study performed in the Segura River basin (Miñano *et al.*, 2003), but higher somatic condition was observed in females in other non-native populations of *G. lozanoi* in the Iberian Peninsula (Lobón-Cerviá & Torres, 1984). Temporal dynamics of the hepatosomatic condition of females was similar to the GSI pattern, increasing at the beginning of the maturation stage and decreasing at the end of spawning. While the hepatosomatic condition of males showed high values at quiescence, HC decreased until the end of the spawning stage. In indeterminate batch spawners as *G. Lozanoi* (Lobón-Cerviá & Torres, 1984; Lobón-Cerviá *et al.*, 1991), oocytes recruitment is continuous during the spawning season and high liver mass (high hepatosomatic condition) could confirm an intense liver activity for vitellogenesis, while in determinate batch spawners or total spawners the hepatosomatic activity decreases during the reproductive cycle due to the completion of vitellogenesis prior to spawning (Dahle *et al.*, 2003; Rinchard & Kestemont, 2003).

Sexual maturity of non-native populations of *G. lozanoi* in the Iberian Peninsula was reached at a young age (Lobón-Cerviá & Torres, 1984; Lobón-Cerviá *et al.*, 1991; Miñano *et al.*, 2003). In rivers of the central area of the Iberian Peninsula, females aged 1+ and of 6.5 cm in fork length were mature (Lobón-Cerviá *et al.*, 1991), while in a previous study in the Segura Basin the shortest mature male and female were 6.2 cm and 5.8 cm in fork length respectively (Miñano *et al.*, 2003). In general, most males and females aged 2+ or above 7.0 cm in fork length are mature (Lobón-Cerviá & Torres, 1984; Lobón-Cerviá *et al.*, 1991; Miñano *et al.*, 2003). In this study, the shortest mature males were 3.55 cm in fork length and females were 4.28 cm long, theirs being the smallest sizes found in the whole Peninsula (Lobón-Cerviá & Torres, 1984; Lobón-Cerviá *et al.*, 1991; Miñano *et al.*, 2003). Sexual maturity can be modulated as a compensatory response to population and environmental factors (Wootton, 1990; Saborido-Rey, 2004). Thus, high relative fecundity together with early maturation may be an effective strategy in the first phases of the invasion process (Bøhn *et al.*, 2004). However, the colonization time in the basin of *G. lozanoi* is very long and these differences could be more related to an adaptation strategy to the environmental factors characteristics of a highly regulated Mediterranean basin than related to its invasion process.

Fecundity and fork length were positively correlated, with larger fish showing a higher number of eggs. Positive relationship between fecundity and age was evident in other non-native populations of *G. Lozanoi* (Lobón-Cerviá & Torres, 1984; Miñano *et al.*, 2003), but no significant differences were found in fecundity at different ages in this study. Assuming 3+ years old fish have a fork length of 7.0 cm in the Segura River (Miñano *et al.*, 2003; Amat-Trigo *et al.*, 2013), absolute fecundity in the Segura basin ranged between 376 and 1149. This fecundity rate was lower compared to other populations of *G. lozanoi* in the Iberian Peninsula at age 3+: 5112 in the Jarama River, 1835 in the Ucero River, 1900 in the Moros River and 3017 in the Matarraña River (Lobón-Cerviá & Torres, 1984; Lobón-Cerviá *et al.*, 1991).In spite of the lower fecundity rate in the Segura Basin, oocyte diameters were higher than in other populations in the Iberian Peninsula, which oocyte diameters of 0.76, 0.76 and 0.73 mm in the Matarraña, Moros and Ucero rivers respectively, while the results of this study showed mean diameter of absolute fecundity between 0.84 and 0.92 mm, which are similar to those previously found in the same basin (0.89 mm in Minaño *et al.*, 2003). These results show that the trade-off is established between egg size and fecundity (Wootton, 1990). Although *G. lozanoi* present life characteristics that are closer to an 'opportunistic strategy'(*sensu* Winemiller & Rose, 1992): small-sized species, early maturation, low fecundity, small oocytes and continuous spawning over an extended reproductive season; and production of larger eggs related to the equilibrium strategy, which is developed in stable habitats such as lentic environments (i.e. lakes) or impounded rivers. In a same species, egg viability and larval size are directly related to oocyte size and, consequently, larger eggs will produce bigger larvae which will be more likely to survive. Egg size also may vary in the same species and year as an adaptive response to the environmental condition future larvae will have to face (Wootton, 1990; Saborido-Rey, 2004).

Somatic condition, hepatosomatic and gonadosomatic indexes can be indicators of energy allocation during reproductive events (Collins & Anderson, 1995; Lambert & Dutil, 1997; Grande *et al.*, 2016). In this study, fish conditions were positively correlated with the percentage of muscle proteins in males and the percentage of ovary proteins in females. Lipid contents in liver and gonads were stable during the reproductive cycle in both sexes. Protein contents were also stable in the liver of males, while proteins contents in the liver of females increased at the beginning of reproduction. These results suggest a synthesis activity in the liver of the yolk and eggshell proteins which are transported to the ovarian tissues for oocyte vitellogenesis and maturation (Wootton, 1990; Aruke & Goksøyr, 2003). Ovarian protein content also increased during the reproductive cycle as a response to the oocyte development and maturation (Nunes *et al.*, 2011; Grande *et al.*, 2016). In females, during gonadal maturation ovarian and liver lipid contents did not vary and protein content increased in both tissues, which could indicate that sufficient energy was available during the spawning season to develop gonads and store energy in the liver. Also, in the absence of other energy sources, feeding during the reproductive season might provide energy for vitellogenesis (Johnson, 2009; Saborido-Rey *et al.*, 2010; Beuchel *et al.*, 2013). The reproduction investment of these fish strongly depends on environmental food availability and provides rapid transport from ecosystem productivity to reproduction, which may allow continuous adjustments of the reproductive effort to food intake (Stephens *et al.*, 2009; Mcbride *et al.*, 2015). In sum, these findings provide evidence of the energy intake of *G. lozanoi* during the reproductive season which is typical of *income breeders*. However, lipid content in muscles decreased significantly during the reproductive cycle in males and females. This variation of muscle lipids could be suggesting energy allocation to reproduction from muscle tissue. The use of muscle tissue as an energy source has been documented for several fish like salmonids (Wiegand, 1996). This pattern could suggest that *G. lozanoi* exhibits an intermediate strategy (*income-capital breeding* strategy) in which energy stored in muscles is also required for reproduction (Henderson & Morgan, 2002; Zudaire *et al.*, 2014).

In this study higher fecundity was related to better somatic and hepatosomatic condition as well as gonadosomatic index. High fecundity and strong recruitment were related to the condition of females in other freshwater fish (Henderson *et al.*, 2000), and low levels of energy reserves can produce skipped spawning in Atlantic cod (*Pseudopleuronectes americanus*, Pleuronectidae) (Kjesbu *et al.*, 1998; Skjaeraasen *et al.*, 2009, 2012). Moreover, on the one hand, somatic condition was positively correlated with batch oocyte diameter, which supports the fact that a good fitness favours larger oocyte sizes. On the other hand, hepatosomatic condition and gonadosomatic index were positively related to egg size of potential and absolute fecundity, suggesting that HC and GSI play an important role during recruitment and development of oocytes. Results also showed a positive correlation of ovarian proteins and lipids with absolute fecundity and oocyte sizes that supports the fact that a greater mobilization of macronutrients to gonads favours recruitment and oocyte quality. Studies of freshwater multiple-spawner cyprinid fish also show an intensive lipid requirement of gonads from the liver to cover the continuous oocyte recruitment during the reproductive period (Rinchard & Kestemont, 2003). However, liver weight may be modified by several factors which can affect the hepatosomatic activity such as temperature, seasons or trophic status (Sáez *et al.*, 1984; Cui & Wootton, 1988; Chellappa *et al.*, 1989; Hung *et al.*, 1990). For example, food availability has an important influence on reserves of protein and lipid in the tissues and there is a food-dependent variation in stored lipid energy which affects the reproductive potential of individual fish (Henderson *et al.*, 1996; Henderson & Wong, 1998; Adam e*t al.* 1999).

In sum, the results obtained concur with the hypothesis considered that populations of *G. lozanoi* showed early maturity, low fecundity and a long reproductive period. Previous studies about species' reproductive traits add multiple spawner small fish with asynchronous oocyte development to reproductive traits (Lobón-Cerviá & Torres, 1984; Lobón-Cerviá *et al.*, 1991; Miñano *et al.*, 2003). All these characteristics are typical of an *income breeding* strategy (McBride *et al.*, 2015). Moreover, in this study, protein and lipid contents on tissues during the reproductive cycle provide some insight into the energy allocation during the reproduction of this species, which suggests that current food intake is the main but not only energy source for this species to reproduce since they also use part of the energy supplement they store. Hence, *G. lozanoi* can be classified as an *income-capital breeder*.

Conclusions

Gonadal activity was similar in both males and females and four reproductive stages were described in the reproductive cycle: quiescence, maturation, spawning and postspawning. Duration of maturation and spawning resulted longer than other non-native populations of *G. lozanoi* in the Iberian Peninsula. Temporal dynamics of fish conditions (somatic and hepatosomatic) determined different condition investments in males and females, being also different to other non-native populations. The somatic condition in males was higher than in females, suggesting a higher energetic cost for females in reproduction. Moreover, high values of hepatosomatic condition in females during maturation and spawning stages reflect an intense liver activity which is related to the vitellogenesis process.

Reproductive traits such as first maturity, fecundity and oocyte size of non-native populations of *G. lozanoi* in the Segura River were also different to other non-native populations, showing shorter sizes of first maturity, which could be part of a compensatory strategy of populations to environmental factors that are common in southern Iberian basins in which high relative fecundity and early maturation are also expected. However, no higher fecundity was recorded in this study compared to other non-native populations of the species. Perhaps, in compensation for such a lower fecundity, oocyte size resulted higher than in non-native populations from others basins in the Iberian Peninsula. Due to that the colonization time in the basin of this species is very long, these differences could be more related to an adaptation strategy to the environmental factors characteristics of a highly regulated Mediterranean basin than related to its invasion process.

Protein and lipid content showed different dynamics in tissues during the reproductive cycle. Stability of lipid contents in the liver and gonads for both sexes and the increment of ovarian and liver proteins in females suggest that most of the energy intake comes from current feeding, which is typical of *income breeders*. However, the decrease in muscle lipids during the reproductive cycle could show a certain degree of energy allocation to reproduction from muscle tissues, which is typical of *capital breeders*. These results suggest that *G. lozanoi* could make use of an intermediate energy strategy between *income* and *capital breeding.*

To sum up, reproductive characteristics of non-native populations of *G. lozanoi* in the Segura River basin showed a certain degree of difference to other populations inhabiting non-native areas in the Iberian Peninsula. However, early maturation and protracted spawning season coincide with the strategy developed in other populations of this species in the Iberian Peninsula. Such a coincidence also confirms multiple spawning and asynchronous oocyte development. These characteristics were typical of the species which pursue an *income breeding* strategy. However, the energy allocation showed in this study revealed an intermediate strategy used by the species which behaved as *income-capital breeders*. The knowledge of emerging invasive species is crucial for conservation management, for instance, because the lack of biological and ecological information on *G. lozanoi* could be hiding potential negative effects on other native species.

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Chapter 6

Is flow regulation modulating the reproductive traits and energy allocation of the non-native *Gobio lozanoi* Doadrio & Madeira, 2004 *in* a Mediterranean basin?

Introduction and objectives

Flow regulation is the most widespread anthropogenic alteration in natural aquatic ecosystems and plays an important role in habitat development, food sources availability and distribution of organisms (Poff *et al.*, 1997, 2010; Bunn & Arthington, 2002). There are many studies that confirm the impact of flow regulation systems (i.e. dams and weirs) on the structure and functioning of rivers and how they affect fish populations worldwide (Poff *et al.*, 1997; Alexandre & Almeida, 2010; Mims & Olden, 2013). Life history traits of fish such as growth, maturation, reproduction or lifespan have been closely shaped by surrounding ecosystem conditions. Ultimately, changes in environmental characteristics may determine variations in life history traits of fish populations (Stearns, 2000; Magalhães *et al.*, 2003; Mims & Olden, 2012).

Fish reproductive strategies include adaptations (anatomical, behavioural and physiological adaptations) that are expressed as a series of traits and which purpose is to achieve the best reproductive tactics for their surrounding environment (Wootton, 1984). Abiotic factors which can modulate reproductive traits are temperature, photoperiod, moon cycles or latitude (Miller & Nummela, 2009). Stream flow is another factor which has been considered an important force shaping fish life-histories, and several flow alteration studies have shown significant effects on reproductive traits as, for instance, changes in the timing of spawning and spawning areas (Cambray *et al.*, 1997; Tan *et al.*, 2010), recruitment failure (Agostinho & Gomes, 1997; Vasconcelos *et* *al.*, 2014) and even changes in reproductive strategies (Tedesco *et al.*, 2008; Mims & Olden, 2013).

Reproductive success will depend on how fish allocate energy during reproduction. The management of energy reserves and energy allocation during the reproduction process determines the reproductive strategy (McBride *et al.*, 2015). Fish species, that can use the energy previously stored in tissues for the development and maturation of reproductive features, have been referred to as *capital breeders* (Henderson *et al.*, 1996; Kennedy *et al.*, 2010). This strategy allows them to separate feeding from spawning grounds in time and space, because each fish can store energy to be used later on (MacKinnon, 1972; Jönsson, 1997). Alternatively, *income breeders* strategists are those species that are not able to store energy and which reproduction success is determined by the environmental resources at the time of reproduction (Stearns, 1989; Wang & Houde, 1994; Peebles *et al.*, 1996). Between these two extreme strategies some species show intermediate characteristics, and features of both energy allocation strategies have been observed (Brown & Murphy, 2004; Ganias, 2013; Mollet *et al.* 2013).

Gobio lozanoi Doadrio & Madeira, 2004 is a small cyprinid, widely distributed in the whole Iberian Peninsula and translocated in the Segura River basin, which has demonstrated a high ability to colonize a broad range of habitats (Doadrio & Madeira, 2004; Doadrio *et al.*, 2011) (see Chapter 2 for more information). In the previous chapter (Chapter 5), the results obtained on the reproductive traits of *G. lozanoi* showed a reproductive strategy similar to other non-native populations of the species in the Iberian Peninsula (Lobón-Cerviá & Torres, 1984; Lobón-Cerviá *et al.*, 1991; Miñano *et al.*, 2003; Vila-Gispert *et al.*, 2005). The non-native populations of the species have been previously classified not only as opportunistic strategists (*sensu* Winemiller & Rose 1992) but also as intermediate strategists since they use strategies ranging from periodic to opportunistic (Vila-Gispert & Moreno-Amich, 2002). Moreover, the study about energy allocation during the reproductive cycle showed a mixed strategy, after which they were considered *income-capital breeders*. This means that *G. lozanoi* mainly uses a simultaneous intake of energy while they are feeding to support reproductive investment, but they also show evidence of a certain degree of use of stored energy as a supplement during the reproductive cycle (see Chapter 5 for more information).

Taking into account that this species can modulate their reproductive traits in different flow regimes, its phenotypic plasticity allowing them to develop a strategy between opportunistic and periodic, and the possibility of being considered *income-capital breeders*, two main hypotheses to test in the present study were raised. Firstly, its reproduction strategy could show inter-population plasticity due to the different flow scenarios that picture it as a species that is closer to opportunists in fluvial sectors with the most unpredictable flow regimes. Secondly, energy allocation mechanisms could be affected by different flow regimes, showing a type of breeding that may be more similar to an *income breeding* strategy in fluvial sectors where flows are more predictable and stable.

There is a scarcity of studies dealing with biology and reproductive traits of *G. lozanoi* (Lobón-Cerviá & Torres, 1984; Lobón-Cerviá *et al.*, 1991; Miñano *et al.*, 2003) and no studies evaluate inter-population variability of energy allocation dynamics during the reproductive cycle and abiotic factors. Moreover, studies like the present study are useful to understand life-history trade-offs and adaptations of breeding strategies to new environments (Glebe & Leggett, 1981; Scarnecchia *et al.*, 2011). The main purpose of this study was to assess reproductive traits and energy allocation during reproduction of *G. lozanoi* inhabiting different fluvial sectors of an invaded Mediterranean basin in the southeast of the Iberian Peninsula. For this purpose, the following objectives have been proposed:

a) To study reproductive and fish condition cycles of the non-native *Gobio lozanoi* in five fluvial sectors of the Segura River Basin.
b) To describe the energy allocation (proteins and lipids content) in tissues of the target fish in different flow regimes.
c) To analyse variability of reproductive traits, fish condition and energy allocation in fluvial sectors with different flow regimes.

Material and methods

Study Area and sampling procedures

This study was conducted in the Segura River basin (drainage area of 18,870 km^2), a highly regulated river located in the southeast of the Iberian Peninsula. The Segura River is affected by a typical Mediterranean climate and the area is characterised by a pronounced spatial and seasonal hydrological variability. Currently, the Segura River is highly regulated in terms of irrigation supply and human demands for water, showing highly modified the natural flow regime with changes in flow magnitude and a reverse seasonal flow pattern in some areas (Vidal-Abarca *et al.*, 1990; Belmar-Díaz *et al.*, 2011) (see Chapter 2 for more information).

Sampled streams were selected following flow regime criteria and were located in different hydrological sectors separated by large dams (Fig. 6.1) (see figure 5.1 in Chapter 5). For the analysis of variability in reproductive traits, five sampling sites ranging from natural to reverse flow regimes were chosen (Fig. 6.1).

In the upper part of the Segura basin, the Tus River (tributary river) (TUS) was chosen to represent natural flow regimes, characterised by seasonal flow patterns with flow peaks in autumn and at the beginning of spring and low magnitudes during the rest of the year, an annual rainfall of 410.4 l/m^2 (cumulative precipitation of 2015) (CHS, 2017) and mild average annual water temperature (15.6°C ± 2.77) (Fig. 6.1). The Camping sampling site (SE1) is located downstream of two little reservoirs (Anchuricas and La Vieja reservoirs, capacity of 6 hm^3 and 1 hm^3, respectively), which are mainly operating to provide a hydro-electric power plant with water. Its flow pattern is similar to a natural regime but with higher daily flow variability, the annual rainfall was 295.8 l/m^2 in 2015 and mild average annual water temperature was also recorded (14.7°C ± 2.15) (Fig. 6.1). Further down, approximately 14 km downstream of the Fuensanta reservoir (capacity of 210 hm^3 and main functions being to provide with irrigation supply and human demands for water, as well as to control flooding) where the Letur sampling site is located (SE2), no flow pattern has been observed and the hydrological regime do not show natural seasonality anymore. Flow is high and constant all year round; total annual rainfall was 342.8 l/m^2 in 2015 and showed cooler average annual water temperature (13.0°C ± 1.59) (Fig. 6.1). The Bajo Cenajo sampling site (SE3) is located right below the Cenajo dam, the largest reservoir of the basin (437 hm^3 of capacity), used mainly for agricultural purposes and flood control. This dam hardly ever releases water in winter but starts having increasing water discharge in spring and summer when water demands for agricultural use are higher (CHS, 2017). These operations cause serious changes in flow patterns, showing a reverse flow regime with high flow levels during the dry summer period and losing autumn flash floods (Fig. 6.1). The 2015 annual rainfall was the highest (431.2 l/m^2) and the average annual water temperature was the coolest (12.5°C ± 1.28) in the study area, probably due to water discharge from the bottom of the reservoir (Olden & Naiman, 2010). The last sampling site, Hoya Garcia (SE4) (located downstream of the above mentioned reservoirs and at the confluence of the Segura and Mundo rivers), is located after a small weir of the hydro-electric power plant. SE4 show 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. Total annual rainfall was the lowest in 2015 (210.8 l/m^2) and the average annual water temperature was the warmest (16.2°C ± 2.01) (Fig. 6.1).

Mean daily flow data were recorded at gauging stations in each hydrological sector (CHS, 2017) and converted to a standard unit of measurement (m^3 s^{-1}). Hydrological variables were calculated with the Time Series Analysis (TSA) within the River Analysis Package (RAP version 3.0.7) (Marsh *et al.*, 2006) for the 1994-2015 flow time period. The following hydrological metrics were chosen as flow alteration descriptors: MDBF (Mean Daily Base flow, m^3 s^{-1}), Fmin (Minimum flow, m^3 s^{-1}), Fv (Flow variability, Q10%-Q90%), HSPeak (Mean of High Spell Peaks, m^3 s^{-1}), HSNum (Number of High Spell, days), MDurRise (Mean duration of Rises, days), MDurFall (Mean duration of Falls, days), Contingency (based on monthly mean daily flow, understood as a periodicity measure between flows achieving higher values when the flow pattern is similar every year) and Predictability (based on monthly mean daily flow, understood as the probability to predict a flow at a given moment) (Table 6.1). The selected metrics explain ecologically significant aspects of the flow regime and its changes in the studied basin.

Environmental characteristics of sampling sites were described in the Chapter 5 (Table 5.1).

Table 6.1. Hydrological variables at each sampling site. Mean Daily Base flow (MDBF, m^3s^{-1}); Minimum flow (Fmin, m^3 s^{-1}); Flow variability (Fv, $Q_{90\%}$-$Q_{10\%}$); Mean of High Spell Peaks (HSPeak, m^3s^{-1}); Number of High Spell (HSNum, days); Mean duration of Rises (MDurRise, days); Mean duration of Falls (MDurFall, days); Contingency and Predictability.

Sampling site	MDBF	Fmin	Fv	HSPeak	HSNum	MDurRise	MDurFall	Contingency	Predictability
TUS	1.880	0.004	-2.527	11.646	130	1.551	1.732	0.093	0.480
SE1	3.414	0.092	-3.042	35.037	70	1.664	1.788	0.109	0.466
SE2	4.923	0.179	-2.167	18.022	82	1.817	1.857	0.074	0.437
SE3	2.567	0.015	-8.052	27.165	50	3.604	5.109	0.178	0.295
SE4	15.434	1.080	-1.443	43.139	80	2.299	2.425	0.106	0.626

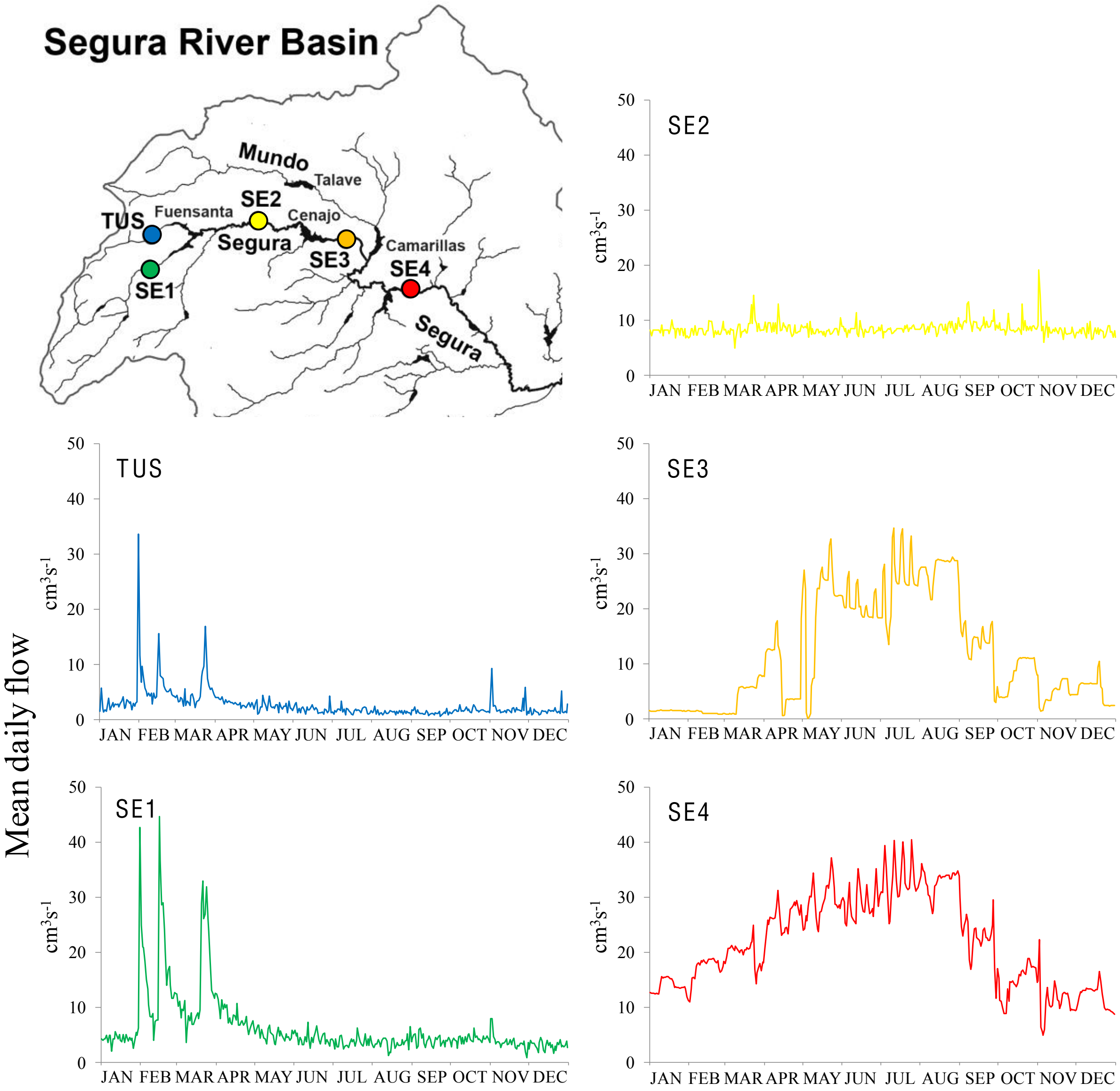

Figure 6.1. In the top left corner sampling sites location in the Segura River basin. In the rest of the figure mean daily flow (cm^3 s^{-1}) of each sampling site in 2015.

Field sampling and laboratory procedures

The methodology that was used to catch and process fish was the same as the one described in Chapter 5.

Fish were collected by electrofishing (CEN, 2003) during a one-year study period (January-December 2015). A total of 2333 *G. lozanoi* were caught (TUS: 437; SE1: 478; SE2: 385; SE3: 485 and SE4: 548), sacrificed in accordance with Spanish regulations and stored at -20°C in the laboratory.

Fork length (L_F ± 1 mm), total and eviscerated masses (M_T and M_E ± 0.1g) and organ masses (hepatosomatic and gonad, M_H and M_G ± 0.001 g) of a subsample of 1982 fish (TUS: 382; SE1: 403; SE2: 365; SE3: 366 and SE4: 466) were recorded. Sex was determined visually (male, female or immature). A subsample of 133 mature specimens (110 females and 23 males) (females, TUS: 20; SE1: 25; SE2: 24; SE3: 17 and SE4: 24; males TUS: 13 and SE4: 10), with fork length ranging from 7.2 and 11.2 cm, were analysed to estimate fecundity, oocyte diameter, protein and lipid content and fish age (see Chapter 5 for more information).

Ovarian development and fecundity were studied using the gravimetric method (Bagenal & Braum, 1978). No significant differences (ANOVA, $P < 0.05$) related to the eggs' position in the gonad were found in egg diameter or egg number and a subsample from the mid-region (5% of the total weight of the gonad) was selected for fecundity and oocyte diameter studies. Image processing program ImageJ v1.80 (available at https://imagej.nih.gov/ij/) was used to count and measure oocytes. Fecundity was analysed at three levels: potential (Fec_{POT}), absolute (Fec_{ABS}) and batch fecundity (Fec_{BAT}). These levels were determined by counting the total number of opaque and vitellogenic oocytes, total number of vitellogenic oocytes and total number of vitellogenic oocytes of the last mode representing size before spawning, respectively (Bagenal, 1978; Murua *et al.*, 2003). Oocyte diameter at each level of fecundity ($Ø_{\text{POT}}$, $Ø_{\text{ABS}}$, $Ø_{\text{BAT}}$) were assessed (see Chapter 5 for more information). Length-frequency distributions were used to study population structure according to each reproductive stage in each sampling site.

Statistical analyses

Sampling site distribution was analysed by a PCA with the above mentioned flow metrics and average temperatures.

Three analyses were carried out to calculate sex-ratio: one for the whole population, another one for each sampling site, and the last one for each reproductive stage at each sampling site. The degree of significance of the obtained results was established in $\chi 2$ at a p-value of $P < 0.05$.

Covariance analysis (ANCOVA) was performed to obtain fish conditions and gonadal activity data (log-transformated data of M_E, M_H and M_G as dependent variables, L_F as the covariate and reproductive stage as factor) as described in Chapter 5. To evaluate differences among sampling sites at each reproductive stage in fish conditions, gonadal activity, fecundity, oocyte diameter and percentage of proteins and lipids in tissues, analyses of variance (ANOVA) were performed followed by Tukey HSD test post-hoc comparisons were made in males and females separately. When data did not show homogeneity of variances, Welch's analysis of variance (ANOVA) followed by T3 of Dunnett for pairwise multiple comparisons were developed. Student's t-test was used to evaluate differences between sexes. Non parametric tests of Kruskal–Wallis H-test and Mann–Whitney U-test were used when data did not fit normal distribution.

Size of first maturity was estimated after running binary logistic regressions (immature-mature individuals) for each sampling site by sex as in Chapter 5. Differences in first maturity among sampling sites were tested using generalized estimating equations (GEE), with binomial errors and the logit link function (Quinn & Keough, 2002; Benejam *et al.*, 2009).

Statistical analyses were performed with the SPSS® software package v. 15.0 and a significance level of 0.05 was accepted.

Results

Flow characterisation

The resulting PCA from hydrological variables and mean temperature explained 51.18% of the variation on axis 1 and 37.70% on axis 2. The first axis was mostly related to flow variability (Correlation coefficient r = -0.89; *P* = 0.043), number of high spell (r = -0.84; *P* = 0.077), rise and fall mean duration (r = 0.96; *P* = 0.009; r = 0.96; *P* = 0.009, respectively) and contingency (r = 0.93; *P* = 0.021). The second axis was positively related to mean daily base flow (r = 0.97; *P* = 0.007), higher minimum flow values (r = 0.97; *P* = 0.006), mean of high spell peaks(r = 0.86; *P* = 0.065), mean temperature (r = 0.59; *P* = 0.297) and predictability(r = 0.77; *P* = 0.130) (Fig. 6.2). The sampling sites distribution on the PCA reflects their flow conditions (Fig. 6.2).

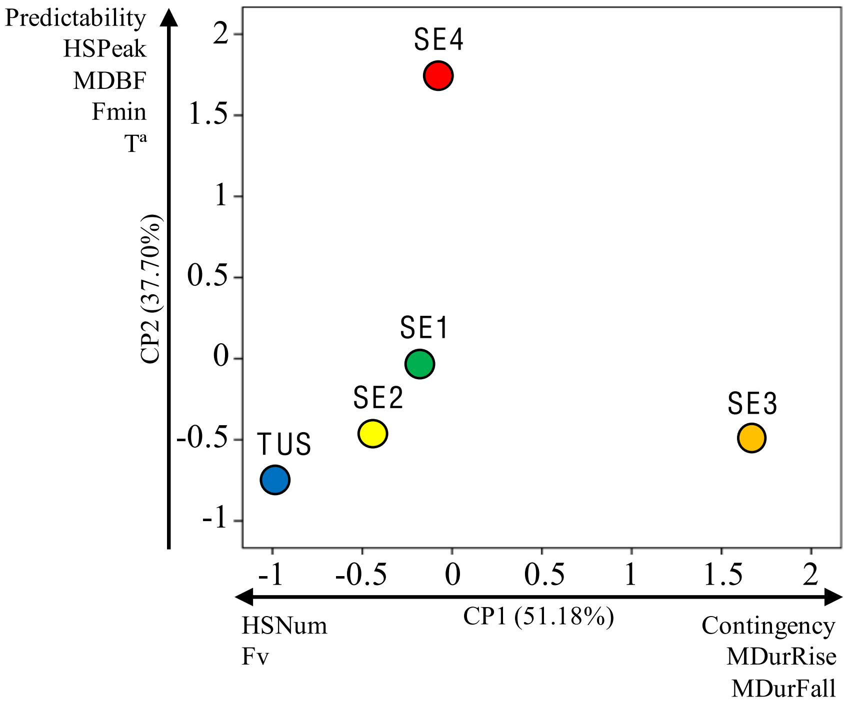

Figure 6.2. Sampling site distribution according the PCA of hydrological variables: Mean Daily Base flow (MDBF m^3s^{-1}); Minimum flow (Fmin m^3 s^{-1}); Flow variability (Fv Q10%-Q90%); Mean of High Spell Peaks (HSPeak m^3s^{-1}); Number of High Spell (HSNum days); Mean duration of Rises (MDurRise days); Mean duration of Falls (MDurFall days); Contingency; Predictability and mean temperature.

Population structure

Gobio lozanoi fish ranged from 1.8 cm to a maximum L_F of 12.3 cm (a male caught in SE3). Total males (L_F 7.6 ± 1.6 cm) were significantly longer than females (L_F 7.1 ± 1.5 cm) (Student's t-test, t = 5.555, $P < 0.001$). No significant differences in L_F between sexes were found in SE1 and SE4 (Student's t-test, $P > 0.05$).

Mean fork length and confidence intervals (95%) of total and in each reproductive stage are shown in Table 6.2. Fork length in females was significantly different depending on the sampling site in the total data (ANOVA, $F_{(4,\ 922)} = 16.989$, $P < 0.001$) and in each reproductive stage (Welch ANOVA, quiescence stage: $F_{(4,\ 85.629)} = 5.267$, $P = 0.001$; ANOVA, maturation stage: $F_{(4,\ 354)} = 6.541$, $P < 0.001$; spawning stage: $F_{(4,\ 218)} = 2.935$, $P = 0.022$; postspawning stage: $F_{(4,\ 144)} = 6.543$, $P < 0.001$). TUS and SE3 showed smaller females and SE1, SE2 and SE4 larger ones. Males showed significant differences among sampling sites in the total data (Welch ANOVA, $F_{(4,\ 308.18)} = 2.571$, $P = 0.038$), being individuals in SE2 larger than in TUS. Analyses in males at reproductive stage only showed significant differences at the postspawning stage with smaller sizes found in TUS and SE1 (Welch ANOVA, $F_{(4,\ 33..772)} = 5.421$, $P = 0.002$).

Table 6.2. Mean values of fork length (± 95% CI) in the total individuals of the populations and at each reproductive stage in each sampling sites in both females and males.

				Mean Fork length		
Sampling site		Total	Quiescence	Maturation	Spawning	Postspawning
Females	TUS	6.53 ± 0.22	6.20 ± 0.46	6.61 ± 0.42	6.61 ± 0.44	6.58 ± 0.44
	SE1	7.51 ± 0.21	7.40 ± 0.34	7.73 ± 0.35	7.14 ± 0.50	7.67 ±0.55
	SE2	7.36 ± 0.21	7.58 ± 0.63	7.26 ± 0.28	7.23 ± 0.46	7.61 ± 0.63
	SE3	6.77 ± 0.24	6.98 ± 0.46	6.71 ± 0.36	6.57 ± 0.61	6.77 ± 0.75
	SE4	7.39 ± 0.16	7.18 ± 0.29	7.29 ± 0.34	7.38 ± 0.29	7.92 ± 0.32
Males	TUS	7.19 ± 0.31	7.62 ± 0.62	7.61 ± 0.58	6.84 ± 0.54	6.84 ± 0.62
	SE1	7.58 ± 0.29	7.29 ± 0.58	8.00 ± 0.61	7.53 ± 0.49	7.09 ± 0.54
	SE2	7.90 ± 0.33	8.20 ± 0.70	7.66 ± 0.47	7.79 ± 0.82	8.27 ± 1.24
	SE3	7.64 ± 0.23	7.47 ± 0.39	7.70 ± 0.28	7.52 ± 0.58	8.10 ± 0.98
	SE4	7.54 ± 0.19	7.40 ± 0.60	7.43 ± 0.30	7.55 ± 0.33	8.23 ± 0.42

Length-frequency distribution by reproduction stages showed differences in spatial scale. At maturation stage, a higher number of fish were caught in all sampling sites and all size classes were well represented. Furthermore, at the postspawning stage the catches were much reduced in all sampling sites and no small sizes were found in SE4 during the reproductive cycle (Fig. 6.3).

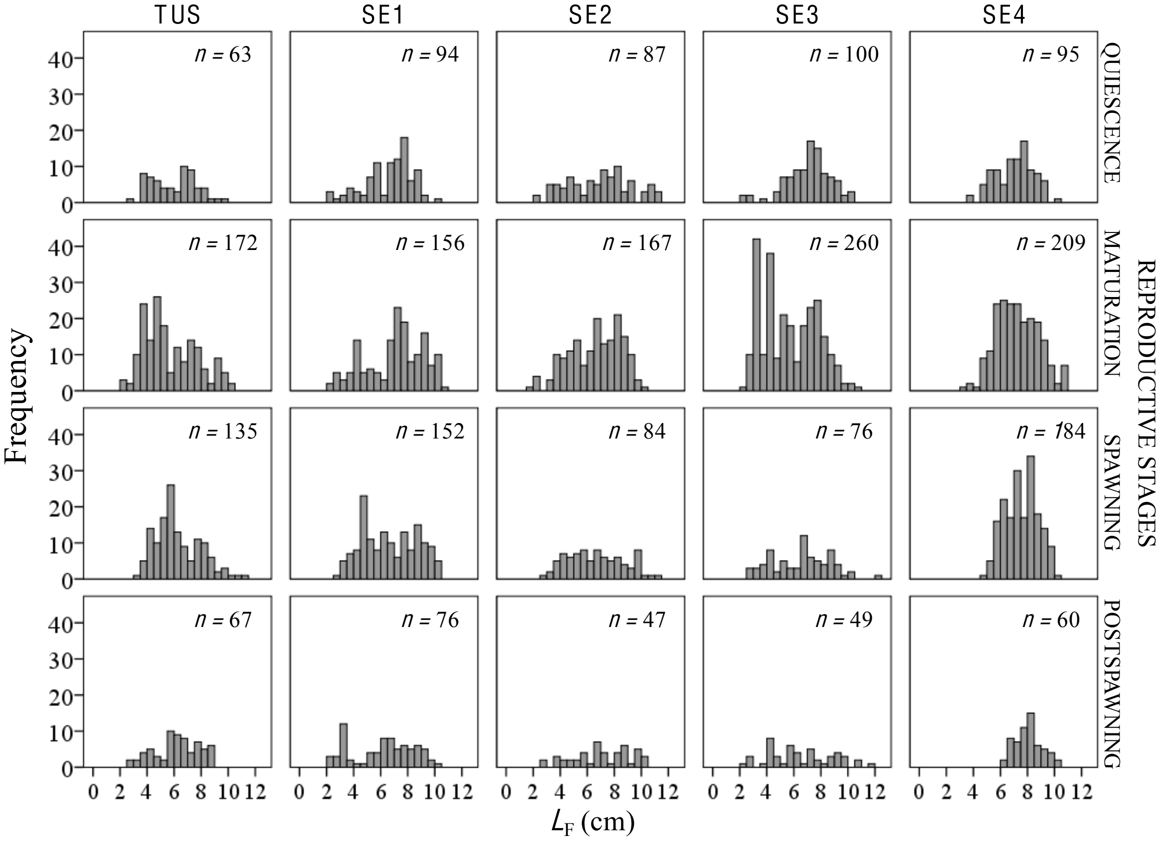

Figure 6.3. Length-frequency distributions of *Gobio lozanoi* populations in each sampling site by reproductive stage. Size intervals of 0.5 cm and number of individuals (*n*).

Reproductive cycle and fish conditions

In the previous Chapter 5, ANCOVAs' predicted M_E, M_H and M_G values were used to study timing of gonadal activity (GSI), determine reproductive stages and, also, to assess somatic (SC) and hepatosomatic condition (HC) dynamics (Fig. 5.2 and Fig. 5.3). Both males and females showed a similar gonadal activity over the reproductive cycle (Fig. 5.2), but GSI was significantly higher in females than in males in all reproductive stages. Fish condition (somatic and hepatosomatic) changes were also found over the reproductive cycle (Fig. 5.3). Somatic condition of males was significantly higher than females' in most reproductive stages, whereas the hepatosomatic condition of females were higher than males during maturation and at spawning (see Chapter 5 for more information).

Significant differences in the gonadal activity among reproductive stages were found when sampling sites were analysed individually for both sexes (Table 6.3). The gonadal activity was significantly different among sampling sites for the total of fish (both males and females) (females Kruskal–Wallis, $\chi^2 = 46.17$, $P < 0.001$; males Kruskal–Wallis, $\chi^2 = 20.09$, $P < 0.001$), SE1 and SE2 populations showing higher GSI values and SE3 the lowest value in both sexes (Table 6.3). GSI comparisons were performed at each reproductive stage and showed significant differences among sampling sites (Fig. 6.4). SE2 showed high GSI values in most reproductive stages in both males and females, while lower values varied among sampling sites (Fig. 6.4).

Table 6.3. Mean predicted M_{E}, M_{H} and M_{G} values by ANCOVA (L_{F} as covariate) in each reproductive stage for both sexes of *Gobio lozanoi*. ANOVA results of comparison of somatic condition (SC), hepatosomatic condition (HC) and gonad activity (GSI) among reproductive stages in each sampling site are showed and significant *P*-values are in bold. * No normalized means data and Kruskal-Wallis analysis of variance.

	Sampling		Reproductive stages					ANOVA	
	site	Total	Quiescence	Maturation	Spawning	Postspawning	df	F	*P*
FEMALES									
SC	TUS	0.651	0.569	0.679	0.683	0.629	3	4.851	**0.004**
	SE1	0.696	0.635	0.736	0.686	0.689	3	5.124	**0.002**
	SE2	0.634	0.664	0.625	0.605	0.665	3	0.908	0.443
	SE3	0.488	0.503	0.480	0.466	0.511	3	0.348	0.791
	SE4	0.583	0.543	0.598	0.567	0.638	3	4.656	**0.004**
HS	TUS	0.043	0.024	0.059	0.051	0.021	3	20.996	**<0.001**
	SE1	0.051	0.036	0.074	0.040	0.025	3	43.062	**<0.001**
	SE2	0.044	0.037	0.041	0.057	0.043	3	1.603	0.199
	SE3	0.025	0.020	0.027	0.029	0.023	3	1.888	0.135
	SE4	0.034	0.028	0.044	0.032	0.026	3	6.309	**0.001**
GSI	TUS	0.179	0.057	0.293	0.205	0.058	3	61.079*	**<0.001**
	SE1	0.225	0.099	0.352	0.212	0.074	3	107.369*	**<0.001**
	SE2	0.196	0.142	0.188	0.323	0.098	3	33.643	**<0.001**
	SE3	0.161	0.084	0.206	0.229	0.058	3	49.019	**<0.001**
	SE4	0.140	0.063	0.234	0.153	0.054	3	146.528*	**<0.001**
MALES									
SC	TUS	0.697	0.641	0.747	0.725	0.586	3	3.409	**0.022**
	SE1	0.745	0.671	0.821	0.756	0.620	3	6.767	**<0.001**
	SE2	0.741	0.779	0.686	0.775	0.776	3	1.920	0.133
	SE3	0.610	0.578	0.621	0.585	0.690	3	1.407	0.249
	SE4	0.638	0.631	0.628	0.636	0.700	3	2.171	0.101
HS	TUS	0.031	0.034	0.036	0.030	0.021	3	1.561	0.206
	SE1	0.030	0.044	0.036	0.025	0.018	3	11.160	**<0.001**
	SE2	0.048	0.053	0.048	0.044	0.044	3	0.437	0.727
	SE3	0.026	0.023	0.028	0.021	0.037	3	4.472	**0.007**
	SE4	0.025	0.033	0.025	0.020	0.032	3	6.724	**0.001**
GSI	TUS	0.040	0.017	0.063	0.041	0.012	3	37.531	**<0.001**
	SE1	0.052	0.020	0.085	0.050	0.014	3	52.044	**<0.001**
	SE2	0.054	0.038	0.057	0.087	0.026	3	11.223	**<0.001**
	SE3	0.040	0.019	0.055	0.042	0.031	3	52.816	**<0.001**
	SE4	0.037	0.016	0.050	0.032	0.017	3	46.862*	**<0.001**

Somatic condition (SC) and hepatosomatic condition (HS) showed significant differences among reproductive stages in both males and females in most sampling sites when analysed individually (Table 6.3). Fish conditions in all females, fish conditions also showed significant differences among sampling sites (somatic condition Welch ANOVA, $F_{(4,327.683)} = 39.991$, $P < 0.001$; hepatosomatic condition Kruskal–Wallis, $\chi^2 = 73.99$, $P < 0.001$), SE1 showing the highest SC and HC values, while SE3 and SE4 showed the lowest in both conditions (Table 6.3). In all males showed significant differences among sampling sites (somatic condition ANOVA, $F_{(4,598)} = 15.564$, $P < 0.001$; hepatosomatic condition Welch ANOVA, $F_{(4,\ 236.262)} = 15.238$, $P = 0.001$), SE3 and SE4 showing the lowest SC and HC values (Table 6.3). Also, significant differences were found when fish conditions were compared among sampling sites at each reproductive stage (Fig. 6.4). In general, SE3 y SE4 populations showed lower SC and HC values at most reproductive stages (Fig. 6.4).

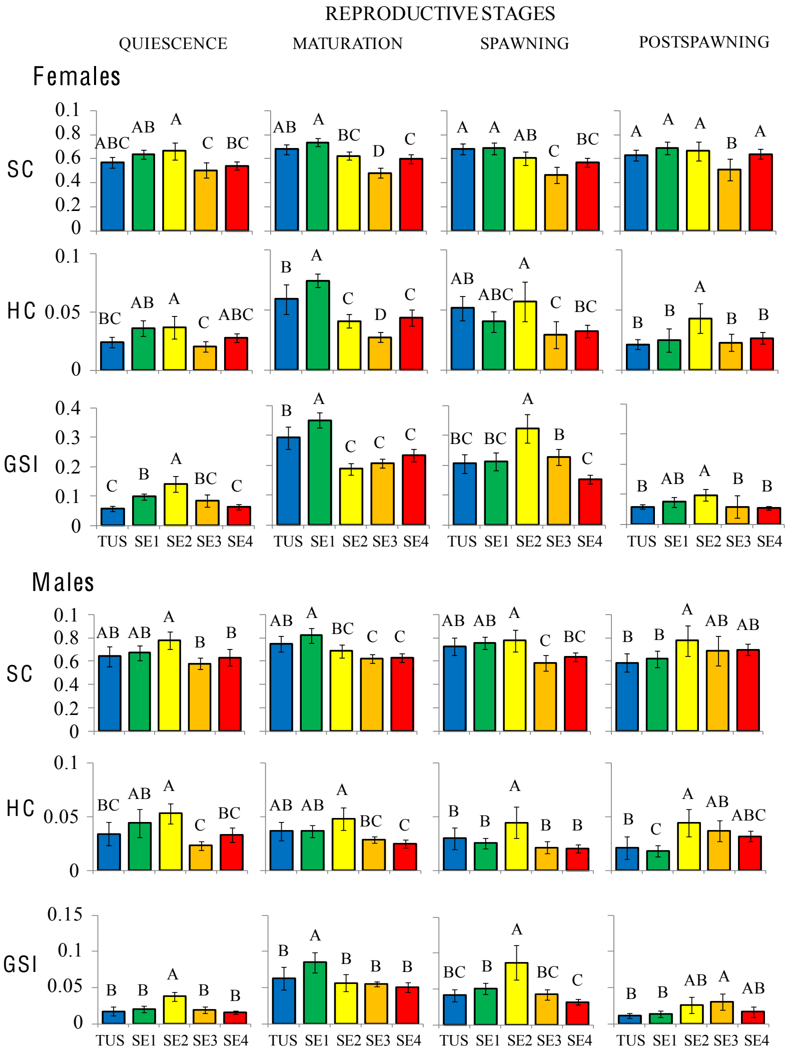

Figure 6.4. Mean predicted M_E, M_H and M_G values (± CI 95%) by ANCOVA (L_F as covariate) by reproductive stage in each sampling site for both sexes of *Gobio lozanoi*. Letters show significant differences (ANOVA, Tukey HDS post hoc tests) among sampling sites.

Reproduction traits

As mentioned in the results of Chapter 5, the overall sex-ratio (696 males, 928 females) was significantly skewed towards females ($\chi^2 = 33.14$, $P < 0.001$), this tendency being observed in most sampling sites (Table 6.4). The analyses of sex-ratio at each reproduction stage were also significantly skewed towards females in several sampling sites and with the exception of SE3 (Table 6.4).

In the previous chapter, length at first maturity showed a range of 3.55-6.26 cm L_F in males and 4.28-6.60 cm L_F in females. However, only significant differences were found in length at first maturity in males (GEE: Wald-$\chi^2_{(4)} = 13.57$ $P = 0.009$), finding significantly longer fish at first maturity in TUS, SE1 and SE2 populations (see figure 5.4 in Chapter 5 for more information).

Table 6.4. Sex-ratio proportions (male: female) of *Gobio lozanoi* by sampling site for every reproductive stage and for the total individuals. Chi-squared tests significant values $P < 0.05$ in bold.

Sampling Site	Reproduction Stages				
	Quiescence	Maturation	Spawning	Postspawning	TOTAL
TUS	**0.45**	**0.59**	0.88	**0.41**	**0.60**
SE1	**0.52**	**0.56**	1.26	0.63	**0.73**
SE2	0.91	**0.51**	0.61	**0.40**	**0.59**
SE3	1.13	0.87	**1.90**	1.43	1.15
SE4	**0.45**	1.14	0.73	**0.46**	**0.75**
TOTAL	**0.67**	**0.75**	0.95	**0.56**	**0.75**

Significant differences were found in potential (ANCOVA, $F_{(1, 4, 39)} = 3.27$, $P = 0.023$) and absolute fecundity (ANCOVA, $F_{(1, 4, 39)} = 2.90$, $P = 0.037$) among sampling sites, SE1 showing a higher number of oocytes and SE3 showing the lowest ones at a given length length (Bonferroni post hoc: $P = 0.023$ and $P = 0.037$, respectively). Only diameter of batch fecundity showed significant differences among sampling sites (ANCOVA, $F_{(1, 4, 37)} = 9.96$, $P < 0.001$). Batch oocyte diameters in TUS and SE1 populations were longer than in SE2, SE3 and SE4 (Bonferroni post hoc: $P < 0.001$) (Fig. 6.5). Fork length was not significant as the covariate with oocyte diameter, which indicates no effect of fish size on egg diameters in the studied fish. No significant differences were found in fecundity and oocyte diameters by age (ANCOVA, $P > 0.05$).

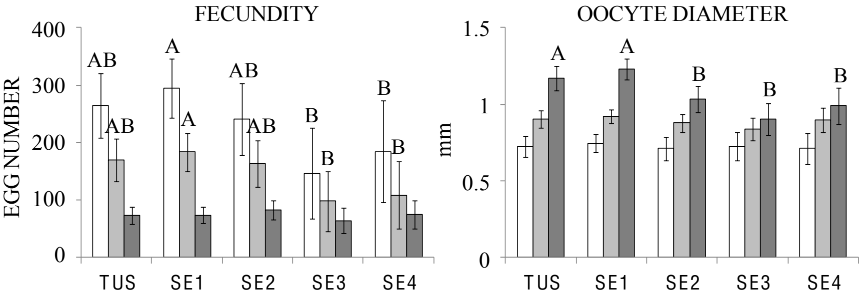

Figure 6.5. Estimated marginal means (by ANCOVA) at 9.0 cm of fork length for oocyte number and diameter of potential fecundity (number of opaque plus vitellogenic oocytes, white bars), absolute fecundity (number of vitellogenic oocytes, grey bars) and batch fecundity (number of oocytes of the last mode representing size before spawning, dark grey bars). Letters show significant differences (ANCOVA, Bonferroni post hoc tests) among sampling sites.

Protein and lipid contents

Percentage of proteins and lipids were analysed in gonads (ovaries and testis), muscle and liver in each sampling site. In females, lipids in muscle decreased from quiescence to spawning and increased until the end of the cycle in all sampling sites (Fig. 6.6), but this trend was only significant in TUS, SE1 and SE3 populations (Table 6.5). Percentages of lipid values in the liver showed a similar pattern, with the exception of TUS and SE1, which did not increase at the postspawning stage, being only significant in SE3 (Table 6.5). In ovaries, lipids slightly increased towards spawning in all the studied populations and an abrupt increase was observed in SE3 and SE4 during the last stage (Table 6.5 and Fig. 6.6). Muscle proteins had different allocation patterns at a spatial level, but were only significant in SE1 where the protein content decreased during the reproductive cycle until spawning stage to later increase in the last stage (Table 6.5 and Fig. 6.6). Protein percentage in liver varied significantly over the reproductive cycle in TUS and SE1 populations (Table 6.5 and Fig. 6.6). In ovaries, patterns of protein percentages were more different among sampling sites, but only SE1, SE2 and SE4 showed significant differences among reproductive stages (Table 6.5 and Fig. 6.6).

Analyses of protein and lipid content in male tissues were performed only in TUS and SE4 populations since there were limitations due to extraction methodology and testis size, and not enough data were collected during the postspawning stage. Only significant differences were observed in percentage of testis proteins over the reproductive cycle in both sampling sites (TUS ANOVA, $F_{(3,10)} = 4.461$, $P = 0.041$; SE4 ANOVA, $F_{(3,6)} = 6.468$, $P = 0.026$) (Table 6.5), showing high values during the quiescence stage, while values decreased during the reproductive cycle (Fig. 6.6).

Comparative analysis of protein and lipid content at each reproductive stage revealed differences among sampling sites. In females, SE3 populations showed higher values of protein percentage in muscles at spawning (ANOVA, $F_{(4,23)} = 9.044$, $P < 0.001$), but lower ones in ovarian tissues in maturation and at spawning stages (ANOVA, $F_{(4,36)} =$

9.667, $P < 0.001$; ANOVA, $F_{(4,21)} = 2.863$, $P = 0.049$, respectively) (Fig. 6.6). SE1 showed the highest values of ovarian protein percentages at postspawning while SE4 showed the lowest ones (ANOVA, $F_{(4,11)} = 3.662$, $P = 0.039$) (Fig. 6.6). TUS showed the highest values of liver proteins at quiescence and spawning stages (ANOVA, $F_{(4,23)} = 8.736$, $P < 0.001$; ANOVA, $F_{(4,23)} = 3.514$, $P = 0.022$ respectively) (Table 6.5 and Fig. 6.6). Differences in lipid percentages were found in muscles at spawning (ANOVA, $F_{(4,23)} = 4.321$, $P = 0.009$), with higher values being observed in TUS (Fig. 6.6). In ovarian tissues, SE3 showed the lowest values at quiescence and the highest during postspawning stages (ANOVA, $F_{(4,19)} = 3.621$, $P = 0.023$; ANOVA, $F_{(4,19)} = 5.348$, $P = 0.014$, respectively) (Table 6.5 and Fig. 6.6). In males, TUS showed higher values of muscle proteins than SE4 at spawning (ANOVA, $F_{(4,6)} = 7.264$ $P = 0.036$) (Fig. 6.6).

Table 6.5. Mean values of percentage of proteins and lipids by tissues in each sampling site by reproductive stages for females. ANOVA results of comparison of percentage contents of proteins and lipids among reproductive stages by tissue in each sampling site are showed and significant *P*-values are in bold.

		Sampling		Reproductive stage				ANOVA	
	Tissue	site	Quiescence	Maturation	Spawning	Postspawning	df	F	*P*
Protein	Muscle	TUS	6.73	7.64	6.97	7.72	3	0.921	0.453
		SE1	6.73	6.87	4.86	6.84	3	8.833	**0.001**
		SE2	8.53	6.73	6.98	7.15	3	1.341	0.316
		SE3	6.30	7.02	8.99	9.55	3	2.214	0.135
		SE4	6.79	6.22	6.01	7.05	3	1.188	0.340
	Liver	TUS	14.62	9.55	12.37	9.93	3	4.986	**0.015**
		SE1	7.29	8.71	10.16	6.23	3	4.841	**0.010**
		SE2	10.85	9.22	9.68	9.13	3	1.006	0.411
		SE3	8.27	10.02	10.14	5.98	3	2.590	0.097
		SE4	9.67	10.20	11.09	8.97	3	0.726	0.566
	Ovary	TUS	9.83	12.42	13.61	9.23	3	3.455	0.042
		SE1	5.33	11.43	15.33	13.66	3	6.051	**0.005**
		SE2	7.70	11.90	13.29	11.93	3	5.543	**0.006**
		SE3	5.50	5.05	7.11	10.10	3	1.065	0.400
		SE4	8.87	9.57	11.69	7.33	3	3.270	**0.043**
Lipid	Muscle	TUS	0.84	0.68	0.47	0.60	3	13.414	**< 0.001**
		SE1	0.80	0.79	0.61	0.71	3	4.327	**0.016**
		SE2	0.97	0.87	0.69	0.77	3	1.691	0.201
		SE3	0.73	0.70	0.67	1.09	3	3.666	**0.044**
		SE4	0.79	0.77	0.65	0.67	3	1.631	0.215
	Liver	TUS	11.99	10.98	6.18	5.31	3	1.313	0.430
		SE1	7.57	7.35	6.14	5.56	3	0.264	0.851
		SE2	5.55	6.68	6.40	10.65	3	1.603	0.260
		SE3	3.93	5.58	4.56	8.58	3	4.006	**0.032**
		SE4	9.45	6.42	4.30	9.48	3	3.424	0.094
	Ovary	TUS	3.74	2.84	3.43	2.78	3	1.578	0.234
		SE1	3.03	3.35	3.87	2.79	3	0.994	0.415
		SE2	3.14	2.67	2.68	2.66	3	0.515	0.677
		SE3	1.36	2.54	3.31	9.09	3	24.410	**< 0.001**
		SE4	2.14	2.81	3.01	4.06	3	4.281	**0.018**

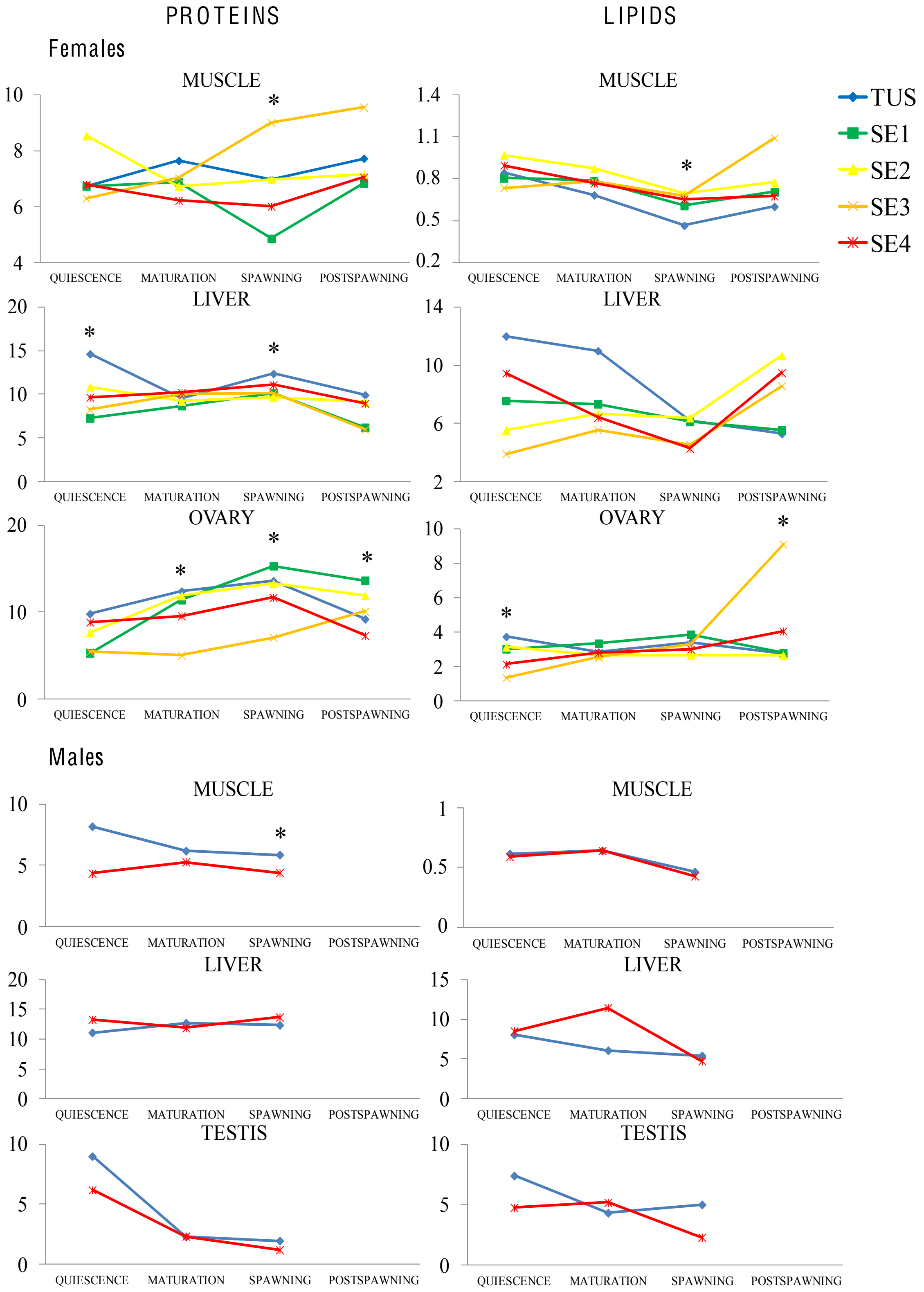

Figure 6.6. Mean values of percentage contents of proteins and lipids in each tissue over the reproductive cycle by sampling site. Asterisks indicate significant differences among sampling site in particular reproduction stage (ANOVA, $P < 0.05$).

Discussion

In this study, the longest fork length observed in the population structure of *G. lozanoi* was 12.3 cm, which was an intermediate value compared to maximum sizes described in other non-native populations in the Iberian Peninsula (ranged between 10.1 and 14.0 cm) (Amat-Trigo, 2017) and lower than the maximum size found in the Segura River basin in a previous study, such fork length being of 14.0 cm in Andreu-Soler *et al.* (2006). In sampling sites located in the most natural flow conditions (TUS), the lowest mean fork length was observed, while in SE4 and SE2 larger males were found. Longer sizes are usually correlated with more stable environments, where abiotic fluctuations as flow peaks are less significant and food sources are more available, so lower mortality rates can be observed (Magalhães, 1993; Harvey *et al.*, 2006). Furthermore, low-flow periods, typical of dry summers in the Mediterranean basins, reduce food and habitat availability and may be affecting growth rates as it has been observed in other Mediterranean cyprinids (Alexandre *et al.*, 2014a,b).

Length-frequency distributions were variable during the reproductive cycle and among sampling sites. On the one hand, bigger catches were recorded at maturation in all sampling sites. Higher fish activity related to an increase in water temperatures at the beginning of the reproductive event may make fish more likely to be caught. On the other hand, only a few fish were collected during the postspawning stage in all sampling sites, but more pronounced in stretches where more varied flow conditions were found. High mortality was found after first-year spawning in other non-native populations of *G. lozanoi* in the Iberian Peninsula (Lobon-Cerviá *et al.*, 1991). However, this species has been classified as iteroparous spawners in most studied populations (Lobón-Cerviá & Torres, 1984; Lobón-Cerviá *et al.*, 1991; Vila-Gisper & Moreno-Amich, 2002). Absence of small-sized fish in SE4 could suggest recruitment failure due to high and unpredictable flow peaks in this area during spawning seasons. However, previous studies carried out in fall at the same sampling site showed that there were more small fish in the same area (Amat-Trigo, 2013) indicating an important and no explanatory variation.

Reproductive cycles of freshwater fish depend on a set of environmental factors and river specialist fish, such as the target species, used to need flow requirements to activate migration processes, gonadal maturation and spawning success (Humphries *et al.*, 1999). Reproduction is related to stream flow, photoperiod and temperature signals (Perkin *et al.*, 2012; Munz & Higgins, 2013; Alexandre *et al.*, 2014a) and there must be optimal conditions for all these variables to coincide in time for gonadal activation to begin. In this study, several intraspecific differences in reproductive traits among populations inhabiting different hydrological sectors have been observed. Temporal dynamics of the gonadosomatic index showed two different patterns in males and females. In both patterns, GSI increased since the middle of February and, one of them increased enormously reaching peak values in April and May in TUS, SE1 and SE4, while the other pattern showed in SE2 and SE3 had a slight GSI increase until June and

July (see Fig. 5.2. in Chapter 5). All populations had slightly decreased until the end of their reproductive period in September. The maturation delay found in SE2 and SE3 could be related to the lack of flow cues in these sectors. Hydrological sector SE2 did not show any high flow peak during the year, and SE3, which is located right below the Cenajo dam, starts to release water in March, while other hydrological sectors show high flows in early February. Thus, the increase in flow stability or reduction of natural flow disturbances, together with a misbalance between temperatures and flow cues may be affecting the beginning of the gonadal activation (Ward & Stanford, 1989; Olden & Naiman, 2010; Alexandre *et al.*, 2014a). Furthermore, the spawning delay observed below the Cenajo dam (SE3) and the disruption of temperature increase (due to hypolimnetic cold water selective releases from the reservoir) may cause gonadal regression or failed oocyte development (Bailly *et al.*, 2008), which could explain lower GSI values observed in fish inhabiting SE3.

Fish conditions (somatic and hepatosomatic) showed differences among different flow regimes during the reproductive cycle. In fact, only in the hydrological sector where flow was constant all year round (SE2), no significant variability was observed for these parameters. No drastic flow events and homogenization of flow conditions may favour stability of fish investment, because fish inhabiting unstable environments with seasonal flash floods may require high levels of energy reserves, such as high somatic and hepatosomatic conditions, a high investment to increase reproductive success (Moyle & Cech, 1996; Vila-Gispert et al., 2000; Alexandre *et al.*, 2014a). On the other hand, the unnatural concurrence of very high flows during the reproductive event (maturation and spawning) implies energy redistribution in fish between survivals in suboptimal environments and reproductive investment (Stearns, 1992), which could explain the lower values of fish conditions and GSI in these hydrological sectors (SE3 and SE4).

Reproductive traits which finally shape reproductive strategies and determine the reproductive success can be directly linked to hydrologic conditions (Mims & Olden, 2012). In this study, the analysed reproductive traits also showed intraspecific variability related to flow conditions. There were a higher number of females in all populations with the exception of those located right below the Cenajo dam, where the number of males was even higher at spawning. Sex-ratio skewed towards females is related to the species' reproductive capacity and environment conditions can affect it (Bruslé, 1982; Dermeche *et al.*, 2009). Thus, very high flows could be increasing female mortality rates after reproductive investment, which could also be observed in low recruitment and catches during the postspawning stage. Variability in sexual maturity is a compensatory population response to different environmental factors (Wootton, 1990; Saborido-Rey, 2004). Many studies relate high mortality in populations to early maturation in order to compensate the decrease observed in adults and maximize the egg production capacity (Aparicio & Sostoa, 1998; Saborido-Rey, 2004). It is expected that areas of high disturbance or extreme environmental conditions and unregulated flow conditions (natural hydrological sectors in this study) are associated with early maturation, which is typical of opportunistic strategies

(Winemiller & Rose, 1992; Mims & Olden, 2013). However, shorter length of first maturation was observed in flow regulated sectors in this study, where natural disturbances are buffered, although some other flow perturbations may be acting as well. Furthermore, higher fecundity and larger oocytes where observed in more natural flow areas, while populations inhabiting more altered flows and with reverse regimes showed a lower number and size of oocytes. The production of larger oocytes could be a compensatory strategy to produce larger larvae which will be more resistant to low-flow stress factors in dry summers, while the production of a high number of eggs can ensure the survival of the species against high mortality rates of eggs and larvae in areas with very variable and unpredictable flows (Wootton, 1990; Saborido-Rey, 2004; Miller & Nummela, 2009). On the one hand, the first hypothesis of this study suggested an opportunistic life-history strategy (small size, short longevity, early maturity, low fecundity, multiple spawnings per year and long reproductive spans) (*sensu* Winemiller & Rose, 1992) in more natural flow conditions, because this strategy is more associated with Iberian native and allocthonous species inhabiting unregulated Mediterranean rivers with strong seasonal flow patterns (Vila-Gispert *et al.*, 2005). On the other hand, characteristics that are closer to those of a periodic life-history strategy (large body size, late maturation, high fecundity and a reduced spawning period) would be expected in more predictable and hydrologically stable environments. However, later maturity and a higher number and size of oocytes were observed in more natural flow sectors; later maturity, higher fecundity and small oocytes were found in the most stable flow sector; and populations with earlier maturity and lower number and size of oocytes were found in hydrological sectors with reverse flow regimes. In general, populations showed intermediate characteristics between opportunistic and periodic life-history strategies as described previously in Vila-Gispert & Moreno-Aminch (2002), and the results were not conclusive enough to establish strong correlations between the life strategy of *G. lozanoi* and flow conditions. In spite of the reproductive strategies being used and reproductive success being directly related to hydrologic conditions (Mims & Olden 2012), the flow effect is not strong enough to drastically change the reproductive strategy of populations of *G. lozanoi* that live in the same basin and may share the same genetic pool. However, the intraspecific variability observed in some reproductive traits suggests a certain degree of effect caused by different flow conditions.

Nutrient acquisition and energy allocation strategies during the reproductive cycle are adaptive as a result of reaching most competitive strategy and reproductive success (Gadgil & Bossert, 1970; Stearns, 1992). Although multiple spawner small fish with asynchronous oocyte development are more likely to use *income breeding* strategies, they often show evidence of some stored energy as a supplement for their egg production (McBride *et al.*, 2015). It is expected that in stable environments, where no natural perturbation constraints food sources availability, species have an *income breeding* strategy, while in seasonal or unpredictable environments, where there is not always food available all year round, species have been adapting to store energy in order to face future events and get more chances of survival or better reproductive investment (Kennedy *et al.*, 2010; Ganias, 2013; McBride *et al.*, 2015). In addition, several species occupying wide latitudinal ranges or inhabiting marginal environments may show intraspecific variations in *capital–income breeding* strategies (Schultz & Conover, 1997; Brown & Murphy, 2004; Ganias, 2013; Wuenschel *et al.*, 2013).

In this study, different flow conditions seem to be affecting energy allocation of populations, showing a decrease in muscle lipid stores in females during the reproductive cycle in populations exposed to drastic flows (very low flows in more natural areas and very high flows below a reservoir dam), while no significant nutrient allocations from tissues were observed in areas with more constant flows (SE2). Higher values of muscle proteins in populations inhabiting below a reservoir could suggest a lower energetic allocation to the reproduction event because higher muscle fitness is necessary to overcome high flows during the reproductive season. Also, this was confirmed by lower values of protein content in gonads during maturation as well as at spawning, which involved lower fecundity and smaller oocytes in this hydrological sector (SE3). As mentioned in the last chapter, all populations seem to meet reproductive energetic requirements from current feeding. However, specific nutrients stored in muscle tissues are involved in the energy allocation needed for reproduction, coinciding with a mixed *income-capital breeding* strategy (see Chapter 5 for more information). Further studies are needed to conclude that *G. lozanoi* turns to store energy for a successful reproduction (*capital breeder* strategy) in seasonal or unpredictable flow environments. However, the results confirm an intraspecific variability related to an energy strategy for the reproductive event modulated by flow conditions.

Environmental conditions have been suggested as modulators of intraspecific variability in the life histories of fish (Tamate & Maekawa, 2000; Heibo *et al.*, 2005; Blanck & Lamouroux, 2007), and stream flow is clearly an important environmental factor that affects the reproductive success of many cyprinids (Munz & Higgins, 2013; Alexandre *et al.*, 2014a). The results of this study have demonstrated that reproduction traits and energy allocation show intraspecific differences in their response to different flows, but populations showed mixed reproductive and energy allocation characteristics which make it impossible to conclude that populations of *G. lozanoi* inhabiting seasonal or unpredictable flow areas show more opportunistic and *capital breeding* strategies than those which live in areas that are hydrologically more stable.

Conclusions

In natural flow sectors, with typical low flow during summer periods in the Mediterranean basin, could be affecting growth rates as smaller individuals were observed, while larger ones were recorded in fluvial sectors without a drastic low flow and a more stable environment. Population structures changed over the reproductive cycle and among sampling sites, showing very few fish at the end of the reproductive cycle, which could suggest high mortality after the reproductive investment in hydrological sectors with a high flow during the reproductive season.

Intraspecific differences were observed in temporal patterns of gonad development, showing a maturation delay in populations inhabiting hydrological sectors where there are no flow cues or such cues may appear later than in natural conditions. Fish conditions also seem to be affected by different flow conditions, showing no relevant variability during the reproductive cycle in sectors where conditions flow are constant but lower values in sectors where high flows coincide with the reproductive event.

Sexes' proportion was skewed towards females except in hydrological sector with a reverse flow regime, where high flows during the reproductive event could show high mortality rates in females due to exhaustion after a hard reproduction investment. In this sector fish reach sexual maturity at early lengths, and lower fecundity and smaller oocytes were recorded, suggesting that water releases from the reservoir have an important negative effect on the reproductive success during the reproductive season.

Populations showed intermediate characteristics between opportunistic and periodic life-history strategies and different flow regimes seem to modulate intraspecific differences, but not according to the initial hypothesis, suggesting that flow effect is not strong enough to drastically change the reproductive strategy of a population at a local and reduced scale.

The energy allocation strategy of *G. lozanoi* populations showed a mixed *income-capital breeding* strategy with intraspecific variability related to flow conditions. However, it cannot be concluded that *G. lozanoi* uses stored energy to achieve reproductive success (*capital breeding* strategy) in seasonal or unpredictable flow environments.

To sum up, results confirm the important effect that flow conditions have on reproductive traits and the energy allocation strategy of *G. lozanoi*, reflected as intraspecific variations in all studied parameters. However, this intraspecific variability was not conclusive enough to classify populations either as opportunistic strategists and more *capital breeders* in unpredictable flow sectors or as periodic strategists and *income breeders* in sectors that are hydrologically stable. Comparative studies of reproductive traits and energy balance are a powerful approach to understanding life-history trade-offs of species, and they may serve as excellent models for studies of plasticity and adaptation of breeding strategies to new environmental conditions in exotic species. Further studies are needed to increase the knowledge about phenotypic plasticity in species that may be potential invaders since the lack of information could be hiding negative effects on other species as well as the environment. Furthermore, ecological information is needed to build useful management and control programs.

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Chapter 7

General conclusions

On the basis of the studies presented in this PhD thesis dissertation, aimed to provide basic information about the population plasticity of the most widely distributed invasive fish, *Alburnus alburnus*, *Lepomis gibbosus* and *Gobio lozanoi*, in the Segura River basin, a highly regulated river basin in the south eastern Iberian Peninsula, the following general conclusions can be drawn:

In relation to the colonization and plasticity in population traits of the *Alburnus alburnus* along longitudinal river gradients:

1. The wide phenotypic variability in population traits enables it to adapt and to establish new populations in highly regulated Mediterranean-type rivers and it seems to be an important factor in its invasion process.

2. *Alburnus alburnus* is widely established in the Segura River basin and its high occurrence confirms the population viability of the species in fluvial stretches, which are suboptimal habitats according to its ecological requirements. Moreover, flow regulation and presence of related lentic shelter habitats seem to be affecting its population status.

3. Size and age population structure was similar to other Iberian populations, but showed shorter size range than native ones. Populations with larger size cohorts and older fish were found in fluvial sectors where residence time was longer, whilst absence of older individuals concurs with earlier stages of invasiveness process, where larger fish, probably after a high reproductive effort, could be suffering higher mortality rates.

4. Abundance, maximum length and growth in *A. alburnus* populations presented a negative relationship with the longitudinal gradient; they showed lower values in

fluvial sectors with higher environmental stress, higher water temperature and conductivity, and poorer ecological status. Moreover, flow regulation has been shown as an important factor which drives population traits, in fact, more abundant populations and faster growth were detected in fluvial sector with medium flow variability and mean daily flow.

In relation to the effects of flow regulation on population size structure of the target invasive freshwater fish:

5. Different flow regimes and presence of artificial-flow habitats derived from anthropogenic impoundment, which act as buffers to drastic flows and provide potential fish refuges, have had effects on fish condition and population size structure of the target species. The observed responses were species-specific in accordance with their ecological guilds.

6. Natural flow regimes typical from Mediterranean basins and the presence of high flow peaks derived to water discharges from reservoirs were important factors shaping the population size structures of target species. Limnophilic or suprapelagic invasive fish, like *L. gibbosus* and *A. alburnus* which are not adapted to overtake the seasonal flash floods, have probably more problems to establish populations. However, homogenization of flow regimes together with presence of artificial-flow habitats derived of impoundment structures, could be favoured their establishment cohabiting with rheophilic freshwater fish whether the latter are translocated or native.

7. The observed size structure of *A. alburnus* supports its population viability in fluvial sectors far from lentic habitats of reservoirs. Although its growth rate was related to flow variability, a significant effect of flow regulation on size-related variables of its populations were not detected. However, populations located next to artificial-flow habitats inside the same hydrological sectors showed higher somatic condition.

8. Reduction of flow seasonality and presence of artificial-flow habitats seem to be decisive factors for the population viability of *L. gibbosus* inside the same hydrological sector. In fact, the nearest locations to artificial-flow habitats showed populations with higher size diversity and elevated occurrence of small individuals.

9. Furthest locations from fluvial stretches with artificial-flow habitats derived of weirs or other impoundment structures were more suitable sites for the rheophilic *G. lozanoi*, showing its size structure a good representation of all size classes with predominance of medium and longer individuals. However, the nearest locations to weirs seem to be more appropriate for the survival of smaller individuals, such it occurred also with the rheophilic native fish *L. sclateri.*

In relation to the reproductive traits and the energy balance strategy of *Gobio lozanoi* inhabiting different fluvial sectors:

10. Reproductive traits of *G. lozanoi* in the Segura River basin showed a certain degree of difference to other non-native populations in the Iberian Peninsula. However, an early maturation and a protracted spawning season concur with the reproductive strategy developed in those Iberian populations, being typical features of species which display an *income breeding* strategy. Nevertheless, the energy allocation observed in our study revealed data that indicate the presence of an intermediate strategy in the species which behaved more as an *income-capital breeder*. Moreover, flow regimes had significant effects on reproductive traits, however, it cannot be concluded about turns in the energy allocation strategy among the hydrological sectors with different flow regimes.

11. Four reproductive stages (quiescence, maturation, spawning and postspawning) were described in the reproductive cycle showing longer maturation and spawning stages than in other non-native Iberian populations. Fish conditions (somatic and hepatosomatic) showed different sex investment, suggesting a higher energetic cost for females in reproduction and also reflecting an intense hepatic activity related to the vitellogenesis process during maturation and spawning.

12. Studied populations showed shorter size of first maturity, lower fecundity and bigger oocytes than other non-native Iberian populations. Due to that the colonization time of the basin by this species is very long, these differences could be more related to an adaptation strategy to the environmental factors characteristics of a highly regulated Mediterranean basin than related to its invasion process.

13. Stability of lipid contents in the liver and gonads for both sexes and the increment of ovarian and liver proteins in females suggest that most of the energy intake during the reproduction come from current feeding, which is typical of *income breeders*. However, the decrease of lipids in muscle during the maturation and spawning could show a certain degree of energy allocation from muscle tissues, which is more related to a *capital breeding* strategy.

14. Intraspecific differences were observed in temporal dynamic of gonad development related to changes in flow regimes, showing delay maturation in populations inhabiting hydrological sectors where no flow peaks are present. Temporal variability of fish condition also seems to be affected by different flow regimes, showing lower values in sectors where high flows coincide with reproductive events.

15. Sex-ratio was skewed towards females except in hydrological sectors with a reverse flow regime, characterised by a high flow level in the dry summer periods and losing autumn flash floods which could carry on high mortality of females due to hard reproduction invest. In this sector individuals reach sexual maturity at early lengths, lower fecundity and smaller oocytes were recorded, suggesting an important negative effect on the reproductive success.

16. Populations showed intermediate characteristics between opportunistic and periodic life-history strategy and different flow regimes seem to modulate intraspecific differences,

however, in disagreement to the proposed hypothesis it suggests that flow regulation effects are not strong enough to change the reproductive strategy at hydrological sector scale.