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EFFECTS OF ENVIRONMENTAL CONDITIONS ON PHENOTYPIC PLASTICITY OF FISHES IN IBERIAN WATERS: LIFE-HISTORY, PHYSIOLOGICAL AND MORPHOLOGICAL TRAITS

Dani Latorre Espeso

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Universitat de Girona

Doctoral Thesis

Effects of environmental conditions on phenotypic plasticity of fishes in Iberian waters: life-history, physiological and morphological traits

Dani Latorre Espeso
2019

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2019

Doctorate program in Water Science and Technology

Thesis supervisor	Co-supervisor	PhD candidate
Dra. Anna Vila Gispert	Dr. David Almeida Real	Dani Latorre Espeso

This thesis is submitted in fulfilment of the requirements to obtain the doctoral degree from the Universitat de Girona

Hereby, Dra. Anna Vila Gispert, of the Universitat de Girona,

CERTIFY:

That this doctoral thesis entitled “Effects of environmental conditions on phenotypic plasticity of fishes in Iberian waters: life-history, physiological and morphological traits", that Dani Latorre Espeso has submitted to obtain the doctoral degree from the Universitat de Girona has been completed under my supervision.

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

Thesis supervisor
Anna Vila Gispert

Girona, 2019

“It is not the strongest of the species that survives,

nor the most intelligent that survives.

It is the one that is the most adaptable to change.”

— Charles Darwin

Després de tot, em trobo escrivint el agraïments de la Tesi doctoral i m'agradaria donar les gràcies a totes les persones que, d'una manera o altra, han format de la meva vida durant aquests anys i sense les quals aquesta Tesi no hagués estat possible.

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Amb el temps les nostres instal·lacions van millorar significativament i vam passar a tenir un laboratori per nosaltres (els dels peixos) al primer pis i un estabulari on tenir en les millors condicions possibles als nostres peixicus. En aquesta nova etapa vaig tenir la sort de conèixer i compartir temps, laboratori i sortides de camp amb la **Maria A**, en **Denis I**, en **Pau Z**, l'**Anna P**, la **Cristina G** i en **Cesc G**, als quals vull donar les gràcies. Recordo que va ser una etapa de molta feina i molt divertida a la vegada. Especialment voldria agrair la seva ajuda, dedicació i suport a la Cristina Galobart. **Cris** gràcies per ajudar-me amb la pesca, el processament de mostres i pels moments de desconnexió que hem compartit i que són tan necessaris. Moltes gràcies per tot. Tot t'anirà genial, ho sé. **Maria**, ya ves parecía que no íbamos a terminar nunca y ya casi lo tenemos esto. Gracias por tu amistad y por tu ayuda en todo momento. Gràcies a tots equipo.

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LIST OF PUBLICATIONS

Part of the results of this doctoral thesis has been published in scientific journals:

- Masó G, Latorre D, Tarkan A.S, Vila-Gispert A, Almeida D Inter-population plasticity in growth and reproduction of invasive bleak, *Alburnus alburnus* (Cyprinidae, Actinopterygii), in northeastern Iberian Peninsula. Folia Zoologica: International Journal of Vertebrate Zoology 65 (2016): 10-14.

- Latorre D, Masó G.; Hinckley A, Rubio-Gracia F, Vila-Gispert A, Almeida D Inter-population plasticity in dietary traits of invasive bleak *Alburnus alburnus* (L., 1758) in Iberian freshwaters. Journal of Applied Ichthyology 32 (2016): 1252-1255.

- Latorre D, Masó G, Hinckley A, Verdiel-Cubedo D, Tarkan A.S, Vila-Gispert A, Copp G.H, Cucherousset J, da Silva E, Fernández-Delgado C, García-Berthou E, Miranda R, Oliva-Paterna F.J, Ruiz-Navarro A Inter-population variability in growth and reproduction of invasive bleak *Alburnus alburnus* (Linnaeus, 1758) across the Iberain Peninsula Marine and Freshwater Research (2018): 1326-1332.

- Latorre D, Masó G, Hinckley A, Verdiell-Cubedo D, Castillo-García G, Vila-Gispert A, Copp, G. H, Cucherousset J, da Silva E, Fernández-Delgado C, García-Berthou E, Miranda R, Oliva-Paterna F.J, Ruiz-Navarro A, Merino-Aguirre R, Serrano J. M, Almeida D Inter-population variability in dietary traits of invasive bleak *Alburnus alburnus* (L., 1758) across the Iberian Peninsula (submitted)

- Latorre D, García-Berthou E, Rubio-Gracia F, Masó G, Galobart C, Merciai R, Almeida D, Vila-Gispert A Inter-population variability in physiology and morphology between wild and captive breeding populations of endangered species *Aphanius iberus* (article in preparation)

LIST OF ABBREVIATIONS

A: Fish ages in years

AaM: Mean fish age-at-maturity

AAS: Absolut aerobic scope

Amax: Maximum age in the fish sample

ANCOVA: Analysis of covariance

ANOVA: Analysis of variance

B: Trophic niche breadth

BC: Body condition

BL: Fish body length

CE: Critically endangered

CEA: Catalogue of Endangered Species

CPUE: Catch per unit effort

D: Density of yolked oocytes

DFA: Discriminant function analysis

E: Endangered

ED: Egg diameter

eTL$_A$: Von Bertalanffy growth equation

eW: Eviscerate weight

FEC: Female fecundity

GI: Growth index

GLMMs: Generalised, linear, mixed models

GLMs: General linear models

GPA: Generalized Procrustes analysis

gW: Gonad (ovary) mass

H': Shannon index

HSD: Post hoc Tukey–Kramer honestly significant difference tests

IUCN: International Union for the Conservation of Nature

K: Fulton's condition factor

LaM: Total fish length at maturity

LC: Least concern

LESPE: List of Wild Species in Special Protection Regime

M: Fish mass

MA: Proportion (from 0 to 1) of mature fish at Age A

MANOVA: Multivariate ANOVA

MMR: Maximum metabolic rate

MO_2: Oxygen consumption rates

MR: Metabolic rate

Na_2SO_3: Sodium sulphite

ND: Without enough data

NE: Northeast

NT: Near threatened

RA: Distance (i.e. scale radius) from centre to the Annulus A

RC: Total scale radius at the time of capture

RI: reproductive investment

S: Prey richness

H': trophic diversity

SD: Standard deviation

SE: Standard error

SL: Fish standard length

SMR: Standard metabolic rate

SR: Sex ratio

SW: Southwest

T*f*: Time swum at the last velocity increment (min),

T*i*: Interval time set

TL: Fish total length

TL_0: Intercept on the length axis from linear regression between all TLC and RC values

TL_A: Total length fish when growth mark (i.e. annulus) A was formed,

TL_C: Total length fish at the time of capture

TL_s: Total length fish at ages

TL_Y: Y-intercept

Ucrit: Critical swimming speed

Uf: highest velocity maintained for a full 20-min period ($cm\ s^{-1}$)

Ui : Velocity increment ($cm \cdot s^{-1}$)

V: Vulnerable

Wg: Gonad weight

X^2: Chi-Square tests

⊿Ob: Microbial respiration

⊿Of: Rates of fish oxygen consumption in mg $O_2\ l^{-1} min^{-1}$

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SUMMARY

Phenotypic plasticity is the adaptive response of a genotype to present different types of phenotypes in response to environmental changes. These adaptive responses can contribute to a specific species occupying a specific habitat or habitats and, in this way, expand its distribution area. These adaptations have been widely studied in fish and it has been possible to confirm the great adaptability that many fish species present to environmental changes. Invasive species represent one of the greatest threats to the conservation of biodiversity and habitats worldwide. These species are considered by the International Union for the Conservation of Nature (IUCN) as the second most important cause of extinction of species, after the destruction of natural habitats.

Bleak *Alburnus alburnus* is a species of invasive fish in different basins of the Iberian Peninsula. This species of cyprinid, however, has not been widely studied and its mechanisms of invasion, establishment and expansion in novel environments are still unclear. On the other hand, Iberian toothcarp *Aphanius iberus* is an endemic and endangered species of the Iberian Peninsula included in Royal Decree 439/1990 of March 30, which regulates the national Catalog of threatened species. The main causes of the reduction of Iberian toothcarp populations are: the destruction of their habitats, the pollution of waters and the introduction of invasive species, such as mosquitofish *Gambusia holbrooki*.

This doctoral thesis expects to study some aspects of the mechanisms of invasion, establishment and expansion of the invasive species bleak in different basins of the Iberian Peninsula and provide more information for a better understanding of its biology and invasion mechanisms. On the other hand, it also aims to compare the morphology, metabolism and critical swimming speed of two Iberian toothcarp populations, one raised in captivity and another one from wild, and to use this

information to apply it to breeding programs in captivity and improve the management of the reintroductions of individuals raised in captivity in their natural habitat.

In the first chapter of this thesis, the plasticity of the fish is put in context as well as the impact of invasive species on ecosystems in general and in the Iberian Peninsula in particular, as well as the effect that phenotypic plasticity may have on to improve the adaptive capacities of the fish to different environmental conditions. The importance of captive breeding of species in danger of extinction such as Iberian toothcarp and the implications that their captive breeding implies is also shown. The second chapter describes the objectives of this doctoral thesis, and its scheme. The third chapter describes the materials and methods used to carry out the different surveys and experiments that have been made in this thesis, describing the sites where the studied populations came from, its environmental typologies, capture, transport and management of the individuals studied, among others. Fourth chapter refers to the different biological traits that were analyzed regarding growth, reproduction and diet such as: total length recalculated at different ages, growth rate, body condition, reproductive investment, length at maturity, age at maturity, sex-ratio, biomass ingested, prey richness, trophic diversity and trophic niche breadth of bleak populations that came from different rivers of the internal basins of Catalonia such as Muga, Fluvià, Cardener and Foix. Given that these populations came from rivers subject to the influence of a typically Mediterranean hydrological regime (variable and unpredictable environments); in the fifth chapter the study of the same biological traits was considered as in the fourth chapter but bleak populations were surveyed in the main rivers of the Iberian Peninsula such as Tagus, Segura, Ebro, Guadiana and Guadalquivir, which present more stable and predictable environmental conditions, and these studied traits were compared with a bleak population from the French Saône river, where this species is native. Finally, the sixth chapter analyzed the phenotypic plasticity in terms of physiology and morphology of two populations of Iberian toothcarp, one of them from a wild environment and the other from captive breeding, the effect of the environment on different biological traits such as the critical swimming capability, metabolism and morphology.

In the fourth and fifth chapters, the results indicated a high variability in terms of the biological attributes and diet of the populations studied. The values of the biological attributes presented characteristics more attributable to the equilibrium strategy (high total length and maturation, low reproductive capacity, greater total length, etc.) in the more rivers with high abundance and with more stable and predictable environmental conditions and values more attributable to the opportunistic strategy (total length and length of small maturation, high reproductive investment, smaller total length, etc.) in those rivers where the environmental conditions are more variable and unpredictable, compared to the results of the native population.

In the sixth chapter, our results showed significant differences in metabolism and morphology among the populations studied. Regarding to critical swimming speed, no significant differences were found. Wild population showed higher values of SMR, MMR and AAS, as well as a more elongated and fusiform morphology that the captive population. These results could be related to environmental factors such as the presence of predators, food availability or intra and interspecific competition among others.

In summary, these results suggest that: 1) Bleak showed a high phenotypic plasticity in terms of life-history characteristics and, therefore, a great ability to adapt to different environmental conditions; 2) The populations of Iberian toothcarp studied differed in physiological and morphological characteristics in spite of sharing the same genetic origin. As a result, this thesis provides valuable information on the ability to adapt to different environmental conditions that bleak presents, when it invades new

territories outside its native range and also very valuable information for the captive breeding programs and reintroductions of Iberian toothcarp populations in its natural habitat.

RESUM

La plasticitat fenotípica és la resposta adaptativa d’un genotip a presentar diferents tipus de fenotips en resposta a canvis ambientals. Aquestes respostes adaptatives poden contribuir a que una espècie determinada pugui ocupar un hàbitat o habitats determinats i d’aquesta manera, expandir la seva àrea de distribució. Aquestes adaptacions han estat àmpliament estudiades en peixos i s’ha pogut constatar la gran adaptabilitat que moltes espècies de peixos presenten als canvis ambientals. Les espècies invasores representen una de les majors amenaces per a la conservació de la biodiversitat i dels hàbitats a nivell mundial. Aquestes espècies estan considerades per la Unió Internacional per la Conservació de la Natura (IUCN) com la segona causa més important d’extinció d’espècies, després de la destrucció dels hàbitats naturals.

L’alburn, *Alburnus alburnus*, és una espècie de peix invasora a diferents conques de la Península Ibèrica. Aquesta espècie de ciprínid però, no ha estat molt estudiada i encara no es coneixen bé els seus mecanismes d’invasió, establiment i expansió en els nous territoris. Per altra banda, el fartet *Aphanius iberus* és una espècie endèmica de la Península Ibèrica en perill d'extinció inclosa en el Reial Decret 439 / 1990 de 30 de març, pel qual es regula el Catàleg nacional d'espècies amenaçades. Les principals causes de la reducció de les poblacions de fartets són: la destrucció dels seus hàbitats, la contaminació de les aigües i la introducció d’espècies invasores com la gambussia *Gambusia holbrooki*.

Aquesta tesi doctoral pretén estudiar alguns aspectes dels mecanismes d’invasió, establiment i expansió de l’espècie invasora *Alburnus alburnus* en diferents conques de la Península Ibèrica i aportar més informació per a una millor comprensió de la seva biologia i mecanismes d'invasió. D’altra banda, també té com objectiu comparar la morfologia, metabolisme i velocitat crítica de natació de dues poblacions de fartet, una

criada en captivitat i una altra de salvatge i utilitzar aquesta informació per aplicar-la a programes de cria en captivitat i millorar la gestió de les reintroduccions d'individus criats en captivitat en el seu hàbitat natural.

En el primer capítol d'aquesta tesi es posa en context la plasticitat fenotípica en peixos així com l'impacte de les espècies invasores sobre els ecosistemes en general i a la Península ibèrica en particular, així com l'efecte que la plasticitat fenotípica pot tenir alhora de millorar les capacitats adaptatives dels peixos a diferents tipologies ambientals. També s'exposa la importància de la cria en captivitat d'espècies en perill d'extinció com el fartet i les implicacions que la seva cria en captivitat comporta. En el segon capítol es descriuen els objectius d'aquesta tesi doctoral, i l'esquema de la mateixa. En el tercer capítol es descriuen els materials i mètodes utilitzats per dur a terme els diferents experiments que s'han realitzat en el transcurs d'aquesta tesi, on es descriuen els llocs d'on provenien les poblacions estudiades, les tipologies ambientals d'on provenien, captura, transport i maneig dels individus estudiats, entre d'altres. En el quart capítol es fa referència als diferents atributs biològics que es van analitzar referents al creixement, la reproducció i la dieta tals com: longitud total retorcalculada a diferents edats, índex de creixement, condició corporal, inversió reproductiva, longitud total de maduració, edat de maduració, sex-ratio, biomassa ingerida, riquesa de preses, diversitat tròfica i amplitud de nínxol tròfic, de diferents poblacions d’alburn provinents de diferents rius de les conques internes de Catalunya com ara la Muga, el Fluvià, el Cardener i el Foix. Donat que aquestes poblacions provenien de rius sotmesos a la influència d’un règim hidrològic típicament mediterrani (ambients molt variables i impredictibles); en el cinquè capítol es va plantejar l’estudi dels mateixos atributs biològics que en el quart capítol però de poblacions d’alburn provinents dels principals rius de la Península Ibèrica com ara el Tajo, el Segura, l’Ebre, el Guadiana i el

Guadalquivir, els quals presenten unes condicions ambientals més estables i predictibles, i comparar els atributs estudiats amb una població d'alburn provinent del riu francès Saône, on aquesta espècie és nativa. Per últim, en el sisè capítol es va analitzar la plasticitat fenotípica quant a fisiologia i morfologia de dues poblacions de fartet, una d’elles provinent d'un ambient salvatge i l'altra de la cria en captivitat, per tal de determinar l’efecte de l’ambient en diferents atributs biològics com ara la capacitat crítica de natació, el metabolisme i la morfologia.

En el quart i cinquè capítols, els resultats van indicar una elevada variabilitat pel que fa als atributs biològics estudiats i a la dieta en les poblacions estudiades. Els valors dels atributs biològics van presentar característiques més atribuïbles a l’estratègia d'equilibri (elevada longitud total i de maduració, baixa capacitat reproductora, longitud total més gran , etc) en els rius amb més abundància, més cabalosos i amb unes condicions ambientals més estables i predictibles i uns valors més atribuïbles a una estratègia oportunista (longitud total i longitud de maduració petita, elevada inversió reproductiva, tongitud total més petita, etc) en aquells rius on les condicions ambientals són més variables i impredictibles, en comparació amb els resultats de la població nativa.

En el sisè capítol, els nostres resultats van mostrar diferències significatives en el metabolisme i la morfologia entre les poblacions estudiades. Quant a la velocitat crítica de natació, no es van trobar diferències significatives. La població salvatge va mostrar valors més alts de SMR, MMR i AAS, així com una morfologia més allargada i fusiforme que la població captiva. Aquests resultats podrien estar relacionats amb factors ambientals com la presència de depredadors, la disponibilitat d'aliments o la competència intra i interespecífica entre d'altres.

En síntesi, aquests resultats suggereixen que: 1) L'alburn va mostrar una alta plasticitat fenotípica en els atributs biològics analitzats i, per tant, una gran capacitat d'adaptació a diferents condicions ambientals; 2) Les poblacions de fartet ibèriques estudiades difereixen en característiques fisiològiques i morfològiques malgrat compartir el mateix origen genètic. Com a resultat, aquesta tesi proporciona informació valuosa sobre la capacitat d'adaptar-se a les diferents condicions ambientals que presenta l'alburn, quan envaeix nous territoris fora de la seva àrea de distribució nativa i també informació molt valuosa pels programes de reproducció en captivitat i reintroducció de les poblacions de fartet en els hàbitats ibèrics.

RESUMEN

La plasticidad fenotípica es la respuesta adaptativa de un genotipo a presentar diferentes tipos de fenotipos en respuesta a cambios ambientales. Estas respuestas adaptativas pueden contribuir a que una especie determinada pueda ocupar un hábitat o hábitats determinados y de esta manera, expandir su área de distribución. Estas adaptaciones han sido ampliamente estudiadas en peces y se ha podido constatar la gran adaptabilidad que muchas especies de peces presentan los cambios ambientales. Las especies invasoras representan una de las mayores amenazas para la conservación de la biodiversidad y de los hábitats a nivel mundial. Estas especies están consideradas por la Unión Internacional para la Conservación de la Naturaleza (IUCN) como la segunda causa más importante de extinción de especies, después de la destrucción de los hábitats naturales.

El alburno, *Alburnus alburnus*, es una especie de pez invasora en diferentes cuencas de la Península Ibérica. Esta especie de ciprínido sin embargo, no ha sido muy estudiada y todavía no se conocen bien sus mecanismos de invasión, establecimiento y expansión en los nuevos territorios. Por otra parte, el fartet *Aphanius iberus* es una especie endémica de la Península Ibérica en peligro de extinción incluida en el Real Decreto 439/1990 de 30 de marzo, por el que se regula el Catálogo nacional de especies amenazadas. Las principales causas de la reducción de las poblaciones de fartets son: la destrucción de sus hábitats, la contaminación de las aguas y la introducción de especies invasoras como la gambussia *Gambusia holbrooki*.

Esta tesis doctoral pretende estudiar algunos aspectos de los mecanismos de invasión, establecimiento y expansión de la especie invasora *Alburnus alburnus* en diferentes cuencas de la Península Ibérica y aportar más información para una mejor comprensión de su biología y mecanismos de invasión. Por otra parte, también tiene como objetivo comparar la morfología, metabolismo y velocidad crítica de natación de

dos poblaciones de fartet, una criada en cautiverio y otra de salvaje y utilizar esta información para aplicarla a programas de cría en cautiverio y mejorar la gestión de las reintroducciones de individuos criados en cautividad en su hábitat natural.

En el primer capítulo de esta tesis se pone en contexto la plasticidad fenotípica en peces así como el impacto de las especies invasoras en los ecosistemas en general y en la Península Ibérica en particular, así como el efecto que la plasticidad fenotípica puede tener para mejorar las capacidades adaptativas de los peces a diferentes tipologías ambientales. También se expone la importancia de la cría en cautividad de especies en peligro de extinción como el fartet y las implicaciones que su cría en cautividad conlleva. En el segundo capítulo se describen los objetivos de esta tesis doctoral, y el esquema de la misma. En el tercer capítulo se describen los materiales y métodos utilizados para llevar a cabo los diferentes experimentos que se han realizado en el transcurso de esta tesis, donde se describen los lugares de donde provenían las poblaciones estudiadas, las tipologías ambientales de donde provenían, captura, transporte y manejo de los individuos estudiados, entre otros. En el cuarto capítulo se hace referencia a los diferentes atributos biológicos que se analizaron referentes al crecimiento, la reproducción y la dieta tales como: longitud total retorcalculada a diferentes edades, índice de crecimiento, condición corporal, inversión reproductiva, longitud total de maduración, edad de maduración, sex-ratio, biomasa ingerida, riqueza de presas, diversidad trófica y amplitud de nicho trófico, de diferentes poblaciones de alburno provenientes de diferentes ríos de las cuencas internas de Cataluña como la Muga, el Fluvià, el Cardener y el Foix. Dado que estas poblaciones provenían de ríos sometidos a la influencia de un régimen hidrológico típicamente mediterráneo (ambientes muy variables e impredecibles); en el quinto capítulo se planteó el estudio de los mismos atributos biológicos que en el cuarto capítulo pero de poblaciones de alburno provenientes de los principales ríos de la Península Ibérica como el Tajo, el Segura, el Ebro, el Guadiana y el Guadalquivir , los cuales presentan unas condiciones ambientales más estables y predecibles, y comparar los atributos estudiados con una población de alburno proveniente del río francés Saône, donde esta especie es nativa. Por último, en el sexto capítulo se analizó la plasticidad fenotípica en cuanto a fisiología y morfología de dos poblaciones de fartet, una de ellas proveniente de un ambiente salvaje y la otra de la cría en cautividad, para determinar el efecto del ambiente en diferentes atributos biológicos como la capacidad crítica de natación, el metabolismo y la morfología.

En el cuarto y quinto capítulos, los resultados indicaron una elevada variabilidad en cuanto a los atributos biológicos estudiados y en la dieta en las poblaciones estudiadas. Los valores de los atributos biológicos presentaron características más atribuibles a la estrategia de equilibrio (elevada longitud total y de maduración, baja capacidad reproductora, longitud total mayor, etc) en los ríos con más abundancia, más caudalosos y con unas condiciones ambientales más estables y predecibles y unos valores más atribuibles a la estrategia oportunista (longitud total y longitud de maduración pequeña, elevada inversión reproductiva, longitud total más pequeña, etc) en aquellos ríos donde las condiciones ambientales son más variables e impredecibles, en comparación con los resultados de la población nativa.

En el sexto capítulo, nuestros resultados mostraron diferencias significativas en el metabolismo y la morfología entre las poblaciones estudiadas. En cuanto a la velocidad crítica de natación, no se encontraron diferencias significativas. La población salvaje mostró valores más altos de SMR, MMR y AAS, así como una morfología más alargada y fusiforme que la población cautiva. Estos resultados podrían estar relacionados con factores ambientales como la presencia de depredadores, la disponibilidad de alimentos o la competencia intra e interespecífica entre otros.

En síntesis, estos resultados sugieren que: 1) El alburno mostró una alta plasticidad fenotípica en los atributos biológicos analizados y, por tanto, una gran capacidad de adaptación a diferentes condiciones ambientales; 2) Las poblaciones de fartet ibéricas estudiadas difieren en características fisiológicas y morfológicas a pesar de compartir el mismo origen genético. Como resultado, esta tesis proporciona información valiosa sobre la capacidad de adaptarse a las diferentes condiciones ambientales que presenta el alburno, cuando invade nuevos territorios fuera de su área de distribución nativa y también información muy valiosa para los programas de reproducción en cautividad y reintroducción de las poblaciones de fartet en los hábitats ibéricos.

1. GENERAL INTRODUCTION

1.1 Phenotypic plasticity in fishes

Since the appearance of the first fishes in the Cambrian period, 500 million years ago, they have evolved giving rise to the great diversity that these vertebrates present today in a wide variety of aquatic environments around the world. Nowadays about 34,000 species of fish are known (Fishbase 2018), which around 15,000 are freshwater species (Tedesco et al. 2017). Freshwater ecosystems cover approximately 1% of the earth's surface and, in this context; freshwater fishes show one of the higher diversity of species and are an important component of global biodiversity (Reid et al. 2013). The scope of the great diversity of freshwater fish species is comparable to the extraordinary geographic reach in its distribution and the high degree of endemism that they show (Lévêque et al. 2008). This colonization of almost any conceivable aquatic environment presented by fishes have been given by adaptations to different environmental conditions at the anatomical, physiological, behavioural and ecological level. For example, Jalili et al. (2015) demonstrated that the morphological differences between different *Alburnus filippi* populations were related to the environmental parameters of the two different habitats where they lived, showing a clear relationship between morphology and environment. Phenotypic plasticity and genetic adaptation have been considered two alternate mechanisms that different organisms can use to adapt to environmental local conditions (Crispo and Chapman 2010). The pressure of natural selection can affect the genetic variation of a population over several generations, resulting in an adaptation of the population to the prevalent local environmental conditions (Cain and Sheppard 1954; Kettlewell 1958; Burger and Lynch 1995; Kawecki and Ebert 2004; Andrew et al. 2010). Adaptive phenotypic change may occur within a generation, producing locally adapted phenotypes without genetic change (Steinger et al. 2003; Ghalambor et al. 2007; Latta IV et al. 2007; Grenier et al. 2016;

Ho and Zhang 2018). Yet, in many systems, both plastic and genetic variation contribute to adaptive phenotypic change (Conover and Schultz 1995; Conover and Baumann 2009). Nowadays it is known that phenotypic plasticity is an important factor in the evolution which is under genetic control and that may or may not be adaptive (Caswell 1983; Pigliucci 2006).

Phenotypic plasticity is defined as the ability of a given genotype to produce different phenotypes in response to varying environments (Scheiner 1993; Pigliucci 2005). Phenotypic plasticity in fishes has been well studied and it is known that facilitates fish's adaptations under certain environmental conditions modying their behavioural, life-history, physiological and morphological traits (Stearns 1989; Scheiner 1993; Agrawal 2001; Hoverman and Relyea 2007; Alexandre et al. 2014; Oufiero and Whitlow 2016; Norin et al. 2016). All responses to environmental cues by phenotypic plasticity are originated at the cellular level and these can be explained by processes that regulate the expression of genes in response to environmental changes (Schlichting and Smith 2002). The type and degree of plasticity are specific to environmental conditions and to the individual traits of organisms; for example, the same trait may be plastic in response to changes in temperature, but not to the change of nutrients, or a certain trait may be plastic in response to temperature while another trait does not (Pigliucci 2006). Plasticity is the result of numerous physiological mechanisms, including transcription, translation, enzymatic and hormonal regulation, that produce local or systemic responses (Whitman and Agrawal 2009). Phenotypic plasticity can be visualized using the "reaction norm" concept. Reaction norm can be defined as a specific function of the genotype that relates to each phenotype with the environment in which it has been produced (Platt and Sanislow 1988). Reaction norms can be reversible or irreversible (Stearns 1989). When environmental change occurs, reaction norms produce a new phenotype with no genetic change. This new phenotype can be genetically fixed if natural selection keeps operating only in the new environmental (Figure 1) (Pigliucci 2006), although other studies have shown that it is not necessary that the environmental stimulus continue for the new trait to be expressed in the following generations.

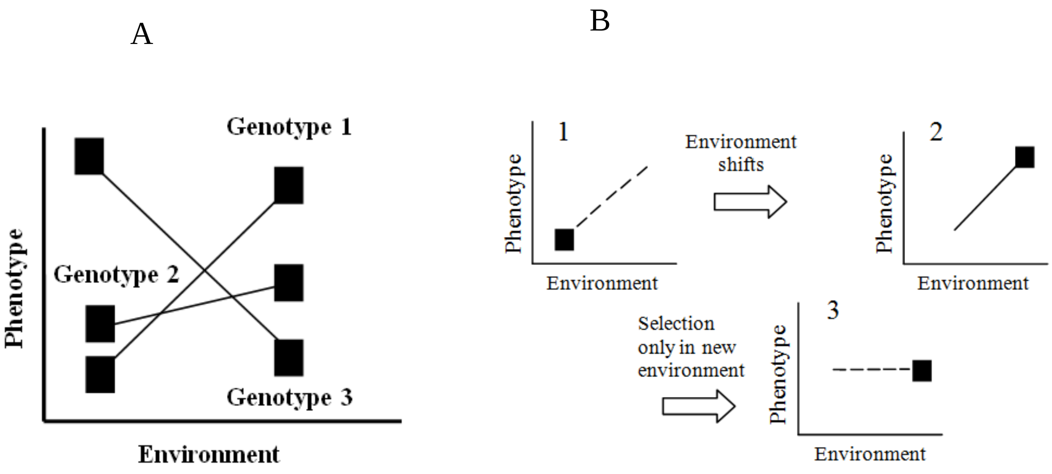


Figure 1. Scheme in relation to: environment phenotype, genotype and reaction norm. A) Genotypic reaction norm illustrating phenotypic plasticiy concept. Lines represent the norm of reaction of each genotype; slopes represent measure of the degree and pattern of phenotypic plasticity. Genotypes 1 and 3 show wide plasticity, but displays opposite patterns in response to the same environment, whereas genotype 2 shows a little plasticity for this trait in this environment set. B) Genetic assimilation of the reaction norm and the phenotypic plasticity. 1 expresses the adaptive potential of the reaction norm in a stable environment. 2 shows the adaptation of the individual to a changing environment thanks to the expression of a new phenotype induced by the reaction norm. At this time there is no genetic change. 3 shows how the new phenotype can be genetically fixed if the new environmental conditions persist over time and how reaction norm can lose plasticity when it is not favoured by natural selection. This figure has been adapted from (Pigliucci 2006).

For example, a study of Waddington (1953) in which after causing a phenotypic change in a particular trait in individuals of *Drosophila melanogaster* induced by an environmental stimulation (e.g. temperature), showed that in a few generations the descendants showed the new trait in the absence of the environmental stimulus inductor of change.

Changing environmental conditions affect the lives of the organisms and their adaptive response to those new environmental conditions. Therefore, the adaptability to these rapid changes in the environment is given by the phenotypic capacity that the different individuals present in certain environmental conditions. Thus, environmental changes act on the phenotypic capacity of individuals, this environmental pressure determines their adaptability and survival and, in turn, these phenotypic changes can influence the evolution of a certain species (Wimberger 1992).

Several studies have demonstrated the wide phenotypic plasticity shown by fishes in life-history traits (Vila-Gispert et al. 2005; Fox et al. 2007; Mérona et al. 2009), swimming performance, swimming capacity and metabolism (Binning et al. 2014; Nelson et al. 2015; Oufiero and Whitlow 2016), morphology (Naspleda et al. 2012; Yavno et al. 2013; Istead et al. 2015), physiology (Holopainen et al. 1997; Maruska and Fernald 2010; Crichigno et al. 2012) among others, in response to environmental changes both biotic and abiotic. Although Darwin's original idea that the history of the organism and diversification is largely a result of common descent and natural selection remains valid, the role that phenotypic plasticity plays in evolution is a subject of wide debate within the scientific community (Pigliucci 2006; Wada and Sewall 2014). Therefore, the adaptive role of phenotypic plasticity are not clearly demonstrated (Ghalambor et al. 2007; Levis et al. 2016; Ho and Zhang 2018), and it is evident the need to continue researching in this field for a greater understanding the role that phenotypic plasticity plays in the evolution of the fish species.

1.1.1 Phenotypic plasticity in life history traits

The life-history traits include those biological attributes related directly or indirectly to reproduction and survival of an individual (Stearns 1989). The patterns of the life-history traits and their coordinated evolution define the life-history of the species (Adams 1980; Rochet 2000; Winemiller 2005). The ability of individuals to produce a sufficient number of offspring to maintain a long-term population in a certain area, determines their survival (Mérona et al. 2009). This ability is related to the life-history traits developed by the individuals, referring to reproduction and growth (Stearns 1992), that will ensure the existence of a new generation. This process is determined by the restriction of the genetic background, which generates the life history of a given species (Stearns 1992; Mérona et al. 2009) and the ability to adapt to environmental changes through phenotypic plasticity (Nussey et al. 2007). Phenotypic plasticity of life-history traits in response to environmental changes has been well documented in fishes (Rodd 1997; Lima et al. 2002; Mims et al. 2010). For example, Mérona et al. (2009) showed changes in life-history traits such as maximum length, absolute and relative length at first maturation, proportion of mature oocytes in ripe gonad, fecundity and mean size of mature oocytes in 14 fish species induced by phenotypic plasticity in response to the construction of reservoirs to adapt to the new environmental conditions. Further, several studies have also shown the wide phenotypic plasticity that present different species of fish in their life-history traits such as age and size at maturity (Roff 1982; Fox et al. 2007; Mims et al. 2010), body condition (Gibbons et al. 1978), reproductive investment and egg size (Pampoulie et al. 2000), back-calculated lengths at age (Top et al. 2018), fecundity (Karjalainen et al. 2016), individual growth rates (Wickins 1987) and sex ratio. In this context, Ospina-Álvarez and Piferrer (2008) found that sex-ratio of some fish species changed depending on temperature. Their work demonstrated a positive correlation between high temperatures and the number of males in some fish species such as *Mendia menidia*; *Odontesthes bonariensis*; *Hoplosternum littorale* and *Poeciliopsis lucida*.

1.1.2 **Phenotypic plasticity in physiology**

Metabolism is the term for a set of biochemical reactions that occur in the cells of living organisms that generate matter and energy to sustain life. Metabolic rate (MR) is the amount of energy expended by an organism in a given time period and this trait provides a view of the physiological state of an animal (Brown et al. 2004). The MR patterns observed in animal populations can be influenced by the level of activity and physiological status, body size, temperature, food intake and anabolism (Norin et al. 2016). Regarding to ectothermic fishes, environment temperature can affect the physiology and oxygen demands (Chabot et al. 2016). In general, physiologists use oxygen uptake when they want to measure metabolic rate of fishes (Chabot et al. 2016). In ectotherms, the most relevant measures of energy metabolism are maximum metabolic rate (MMR), standard metabolic rate (SMR), and absolute aerobic scope (AAS) (Rosewarne et al. 2016), which define metabolic phenotype of an individual (Metcalfe et al. 2016). MMR represents maximum short-term energy output for demanding activities, usually measured as peak aerobic output during forced locomotion (Biró and Stamps 2010), and is associated to the maximum rate at which oxygen can be transported from the environment to mitochondria. Standard metabolic rate (SMR) is the minimum metabolic rate necessary to keep an ectotherm alive at a certain temperature in the post-absorptive period, below this rate the biological function is impaired (Brett and Grover 1979; Priede 1985), and is associated to the minimum rate at which oxygen can be transported from the environment to mitochondria. Subtracting

SMR from MMR provides a measure of absolute aerobic scope (AAS) and represents the aerobic physiological capacity of fish to perform several oxygen-demanding processes simultaneously (Clark et al. 2013).

Differences in MR have been evidenced between individuals of the same and different fish species (Killen et al. 2010; Binning et al. 2014; Auer et al. 2015). There may be a variation between individuals of the same species for metabolic rates after accounting for variation due to factors as temperature and body size in stable conditions (Millidine et al. 2009; Norin and Malte 2011; Killen et al. 2012). The individual variability of SMR found in fish species, is related to the energy demand of vital organs and the availability of resources (Auer et al. 2015). Although there are fewer studies about individual variation of MMR than SMR, it seems to be that intraspecific variation of MMR and SMR is similar (Metcalfe et al. 2015). Therefore, regarding to individuals of the same fish species, variability in SMR and MMR is related to environmental factors such as feeding, temperature, oxygen availability and water flow (Killen et al. 2010; Metcalfe et al. 2015; Auer et al. 2015). For example, Binning et al. (2015) demonstrated differences in MMR of juvenile individuals of *Acanthochromis polyacanthus* reared in different water flow regimes, showing a higher value for individuals reared in a stronger water flow compared to individuals reared in a calm environment.

Further, it is known that MMR is related to swimming performance, cardiac function and phenotypic flexibility towards environmental perturbations (Norin and Clark 2016). Individual SMR can also be related to behavioural traits, such as dominance, risk taking, growth rate, and amount of food intake (Metcalfe et al. 2015). For example, Van Leeuwen et al. (2012) demonstrated that changes in SMR of juvenile coho salmon *Oncorhynchus kisutch* individuals were related to the amount of food consumed. At the interspecific level, metabolic rate differences are generally related to lifestyles (Figure 2) (e.g benthic *v*. pelagic). For example, Killen et al. (2010) demonstrated that the metabolic level is higher in pelagic than benthic fish species (Figure 2).

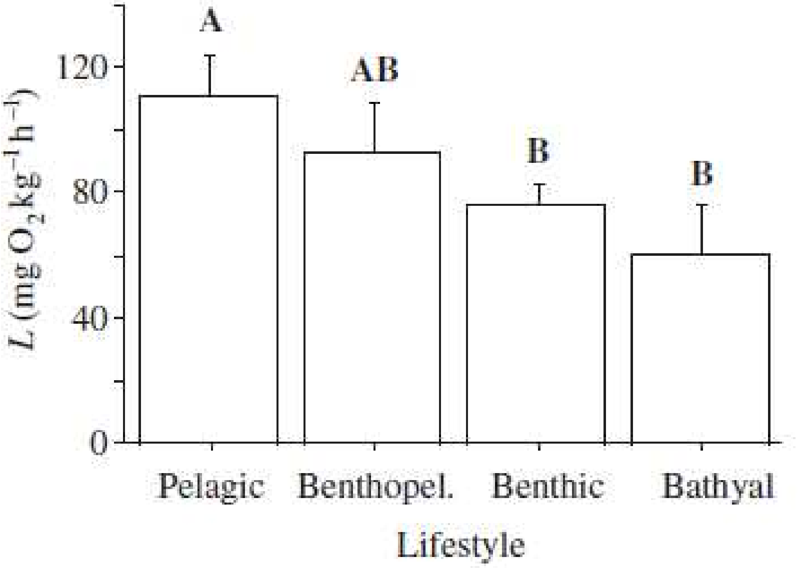


Figure 2. Effect of life style on metabolic level (L). This figure has been adapted from Killen et al. (2010).

At last, all this information on the metabolic phenotypes of fish species both at inter and intraspecific level shows the great variability of factors that may be affecting the metabolic phenotypes of fish.

1.1.3 **Phenotypic plasticity in morphology**

From the beginnings of the first ichthyological studies carried out by Peter Artedi (1705-1735) and Carolus Linnaeus (1707-1778), the first taxonomic studies and classifications in fishes were based on the morphological characteristics presented by the different species. Although phylogenetic advances are helping to reclassify the fish species (Betancur et al. 2017), main knowledge on systematics and evolutionary relationships of fish species, as well as the wide information about their diversity, is based on the study of their morphology (Arratia and Johnson 2015). Morphology is the result of genetic and environmental influences and the environmental component of variation is commonly referred to as phenotypic plasticity (Vøllestad 1992). Several studies have demonstrated the importance of factors that affect morphological plasticity such as: diet, water flow, presence of predators, intra- and interspecific relationships, temperature and food abundance (Stabell and Lwin 1997; Whiteley 2007; Wilcox and Martin 2006; Weber et al. 2012; Alexandre et al. 2014; Jalili et al. 2015; Black et al. 2017). In this context, Alexander et al. (2014) showed morphological differences between two populations of *Luciobarbus bocagei* that were related to the type of hydrologic regime of the environments from which they came (permanent *vs* temporary). The population from a permanent hydrological regime showed more fusiform body shape, narrower head and caudal peduncle, lower body condition and longer and higher pectoral and dorsal fins, when compared with the population from the temporary hydrological regime. Other studies have demonstrated that predator presence induced significant morphological changes in fishes. According to Domenici et al. (2008), individuals of C*arassius carassius* showed significant changes in morphology induced by the presence of the predator *Esox lucius* such as a deep body and a high percentage of muscle mass increasing their escape locomotor performance (Figure 3).

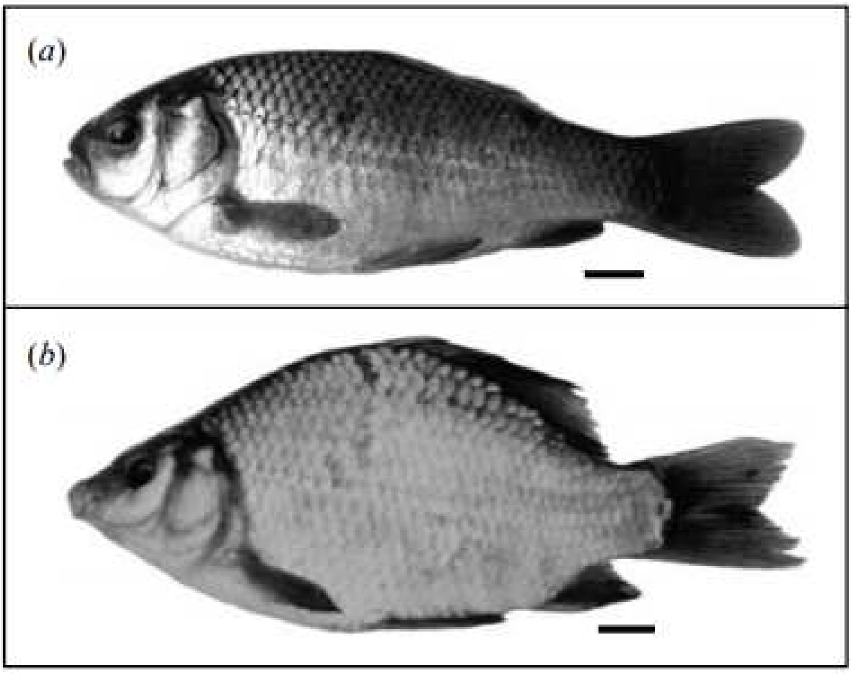


Figure 3. Crucian Carp (*Carassius carassius*) morphological differences induced by presence of predators (a) Shallow-bodied morph from a predator-free pond and (b) deep-bodied morph from a pond with predators. Scale bars, 10 mm. This figure has been adapted from Domenici et al. (2008).

Rapid adaptation to environmental changes has been considered advantageous for fish species, but some studies have revealed data that contradicts this statement. For example, Araki et al. (2007) demonstrated how a few generations of domestication of rainbow trout *Oncorhynchus mykiss* can hinder the reproductive success when they are returned to the wild. Although breeding endangered fish species in captivity is a conservationist strategy, using wild fish parents to restore wild populations and to protect them, it is not clear that it was a well strategy to fight against extinction of endangered species (Araki et al. 2007; Fraser 2008; Mameri et al. 2018).

Phenotypic plasticity shown by fishes have allowed them to survive to environmental changes, adapt and spread for different aquatic environments around the world. Therefore, phenotypic plasticity can be an advantage for individuals to face new situations, which also enables them to invade and settle in a new environment outside their native range (Agrawal 2001; Yeh and Price 2004).

1.2 Invasive fish species on freshwater ecosystems

Freshwater ecosystems have suffered serious impacts due to the introduction of invasive species by humans (Ricciardi and Macisaac 2011). The term invasive species is often confused with exotic species (Colautti and Macisaac 2004). An exotic species is an animal, plant or microorganism species, which is introduced outside its native range distribution, where it does not exist naturally (Panawala 2017). An invasive species is an exotic species, which causes environmental and economic harm to the ecosystem where it has been introduced (Beck et al. 2008; Panawala, 2017). To become invasive, a species must pass some steps successfully (Figure 4).

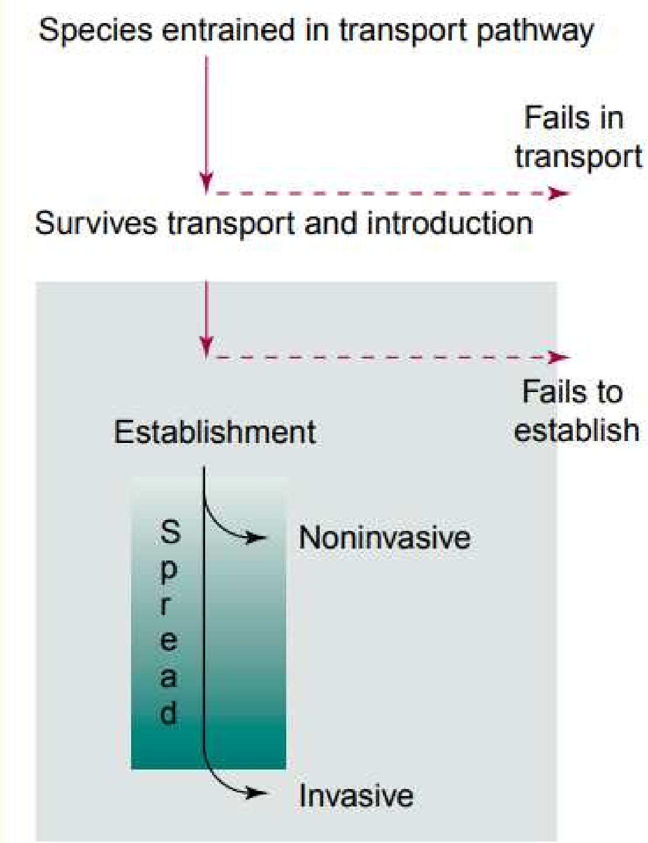


Figure 4. Scheme of Invasion Process. Transitions that non-native species must overcome to continue in the invasion process and become invasive species. This figure has been adapted from Kolar and Lodge (2001).

The impact of invasive species on native species, communities and ecosystems has been widely recognized for decades (Lodge 1993; Kevin and Colin 2003), and invasive species are currently considered an important component of global change (Vitousek et al. 1997). The economic impact of these species is evident; the economic costs of invasive species are estimated from millions to billions of dollars annually (Pimentel et al. 2000). In addition to the economic impacts, invasive species have serious consequences for biodiversity (Mcneely 2001; Ricciardi and Macisaac 2011). In fact, they are considered by the International Union for the Conservation of Nature (IUCN) as the second most important cause of species extinction, after the destruction of natural habitats. Several studies have demonstrated the impacts of invasive species on native species and on the structure of the community (Miller 1989; Witte et al. 1992; Wilcove et al. 1998; Parker et al. 1999; Sala et al. 2000) and ecosystems (Vitousek and Walker 1989; Ricciardi and Rasmussen 1999; Levine et al. 2003; Charles and Dukes 2007). Freshwater ecosystems are the most vulnerable habitats to invasion of species (Cohen and Carlton 1998) and more and more the number of introductions, accidental or intentional, of invasive species increases (Casal 2006). In the Iberian Peninsula, exotic species have flourished, mainly released for angling purposes or biological control, but also as a result of accidental introductions from aquaculture facilities (Maceda-Veiga et al. 2013). The acclimatization of these species is one of the most important negative factors that affect the survival of endemic species (Elvira 1995). As a result, in the Iberian Peninsula the majority of native fish species are threatened (Elvira 1996).

Over the last few decades, the growing problem of invasive species has led to the development of a series of predictive models based on the biological attributes of the introduced species and on the biotic and abiotic characteristics of the environments where they have been introduced (Kolar and Lodge 2001; Vila-Gispert et al. 2005). For the development of these predictive models, the biological attributes that have been taken into account are body size, growth rates, life-history strategy and ecological tolerances that have allowed the invasive species to establish successfully (Copp et al.

2005). Opportunistic life-history strategies such as "r”, are predicted to be favoured in the initial stages of the establishment of a new species and their spread, due to a low density of individuals and in many cases, the high availability of food. Non-native species can succeed in unpredictable and unstable environments. An introduced species that is expanding its range of distribution, it is expected that it will present features such as early maturation and a high inversion of energy in reproduction (MacArthur and Wilson 1967; Taraghi et al. 2011) (Figure 5). Once the species has been established, these features do not necessarily have to be favoured. At this time, success may depend on biological features that allow introduced species to succeed in a more competitive environment, the "K" vital strategy (MacArthur and Wilson, 1967; Taraghi et al., 2011) (Figure 5). In this second stage, it would be necessary to observe a greater investment of energy in the care of offspring and, a decrease in the investment in reproduction (Bohn et al. 1998).

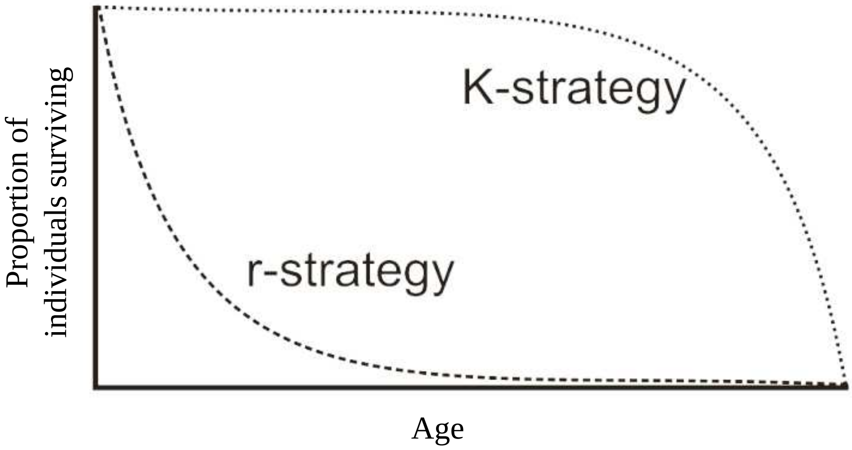


Figure 5. "r" and "K" strategies according to MacArthur and Wilson theory. This figure has been adapted from Taraghi et al. (2011).

1.2.1 Bleak in Iberian freshwaters

Bleak colonization and expansion in the Iberian Peninsula represents a threat to Iberian endemic species (Clavero and Garcia-Berthou 2006) because it is known that bleak can easily hybridize with other cyprinids (Blachuta and Witkowski, 1984; Crivelli and

Dupont 1987). Specifically, there are hybrids with species of the genus *Squalius* (Wheeler 1978; Witkowski and Blachuta 1980; Kammerad and Wuestemann 1989) as the calandino *Squalius alburnoides* (Almodóvar et al. 2012). More serious are the hybridizations with the bermejuela *Achondrostoma arcasii*, included in the List of Wild Species in Special Protection Regime (LESPE), and the jarabugo *Anaecypris hispanica*, which is included in the category of Endangered within the Spanish Catalogue of Endangered Species (CEA). In addition, it is known that interspecific competition via aggression (Vinyoles et al. 2007), has achieved to displace some native species of the Iberian Peninsula from their natural distribution ranges (Almeida and Grossman 2014; (Ribeiro and Leunda 2012). Bleak has shown great variability in its biological attributes which indicates that this species can display a wide phenotypic plasticity in response to the environmental conditions of each type of habitat.

Bleak is often used as a forage fish for angling piscivorous species such as the american perch *Perca flavescens* or rainbow trout *Oncorhynchus mykis*s (Bruno and Maugeri 1995), which could be a factor to be taken into account to explain its presence in the rivers of the Iberian Peninsula where sport fishing is practiced. It has been observed that the presence of bleak is often associated with the introduction of non-native larger predators such as *Esox lucius*, *Micropterus salmoides* (Elvira and Almodóvar 2001), *Sander lucioperca* and *Silurus glanis* (Carol et al. 2003). In some regions of Europe, it is exploited commercially but it is a species not much appreciated by anglers. Furthermore, bleak has great variability depending on the habitat it occupies. For example, Erdoğan and Koç (2017) showed that the length-weight relationship and its reproductive period could vary depending on their geographical distribution.

The establishment of bleak and other invasive species in the Iberian Peninsula has been favoured by the poor conservation of river ecosystems and by the high regulation of river's flow by humans (Elvira et al. 1998). The construction of dams, reservoirs and the excessive extraction of water, in order to regulate rivers, has led to a progressive change of rivers towards more lentic environments. It has been observed that this alteration has occurred at the same time as the expansion of bleak (Elvira et al. 1998). Vinyoles et al. (2007) suggested that in the Iberian Peninsula, there is a relationship between the expansion of bleak and the construction of dams. The fact that human being has made changes along the rivers and has favoured the emergence of more lentic environments is very relevant when we consider that this can favour the spread and establishment of certain invasive species to the detriment of the endemic species of the Iberian Peninsula.

The first citation of bleak in a river of the Iberian Peninsula was in June of 1992 in the Noguera Ribagorçana, a tributary of the Ebro River (Figure 6) (Vinyoles et al., 2007). Four years later, it was found in five more tributaries of this basin: Cinca, Guadalope, Jalón, Matarranya and Segre (CHE 1997). In the basins of the eastern Pyrenees, the first individual of bleak was detected in the Muga River in 1997 (Cardona et al. 2002). In 1999, the presence of this species was mentioned in two more basins of the Iberian Peninsula: to the north of the Duero basin, specifically to the Tormes river (Velasco 2005) and also to the south of the Guadalquivir basin, in Portugal, where it has increased its distribution to the border with Spain (Pérez-Bote et al. 2004). To the east of the Iberian Peninsula, bleak expanded throughout the Ebro basin and Mediterranean rivers such as Júcar, Turia and Mijares (Doadrio 2001). In 2002, the presence of bleak in the Fluvià river (eastern Pyrenees basin) was also mentioned, and it is thought that due to a particular introduction (Vinyoles et al. 2007) (Figure 6). It was also introduced in several reservoirs. Carol et al. (2003) pointed out that this species was already in reservoirs of the Ter, Foix and Llobregat rivers, in the eastern Pyrenees. During 2003, this species was also cited in the Arga river, a tributary of the Ebro (Vinyoles et al. 2007) (Figure 6). Later, its presence was also cited in the Segura river basin (Andreu-Soler et al. 2004). Then, it was detected in two reservoirs of the Guadiana basin in the Spanish zone (Perez-Bote et al. 2004) and in three of the Duero basin (Velasco et al. 2005). In 2005, this species was also found in the Tagus river (Vinyoles et al. 2007) (Figure 6).

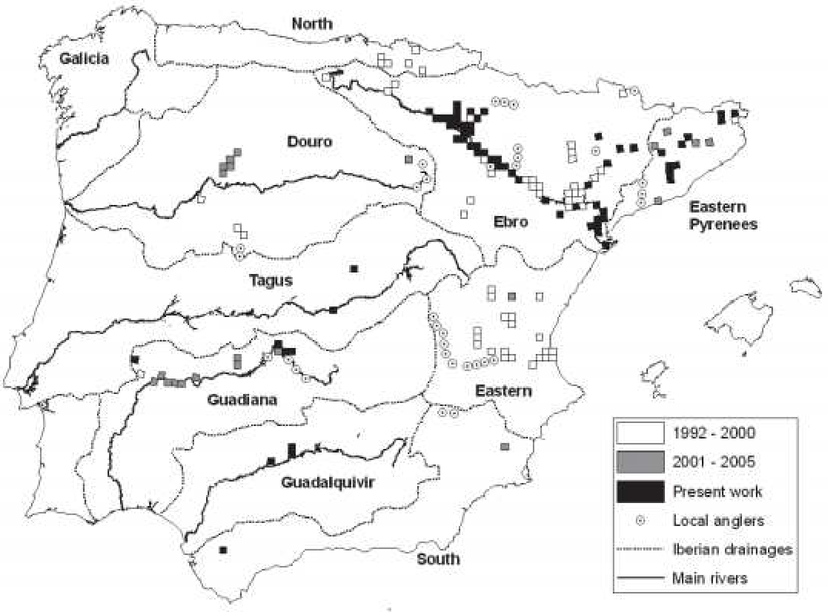


Figure 6. Distribution of bleak *Alburnus alburnus* in the Iberian Peninsula in 2007. This figure has been adapted from Vinyoles et al. (2007).

1.3 Fish species in Iberian waters

Iberian Peninsula borders with Mediterranean Sea on the east and southeast, with the Atlantic Ocean on the west, south-west and north-west, and with Pyrenees on the northeast. This geographical isolation, in terms of freshwater ecosystems, its evolutionary history and the characteristics of its river systems, have caused the Iberian Peninsula to have a large number of freshwater endemic fish species (Doadrio et al. 2011). An endemism is a taxon that has a restricted distribution, which is only present in a certain area of the world (Morrone 2008). The number of fish species that inhabit freshwater ecosystems of the Iberian Peninsula is 89 species (Doadrio et al. 2011). Of these, 61 are native species and of these, 51 are strictly freshwater species. In Iberian Peninsula there are also 10 species of estuarine habits and/or migratory. Of the 51 strictly freshwater species, 41 are endemic species, which suppose more than 80% of the freshwater species of the Iberian Peninsula. In Iberian freshwater ecosystems, also inhabit 28 exotic species (Doadrio et al. 2011). Of the 61 native species, 57 of them, are included in some category of threat (Figure 7). Some of these species are *Luciobarbus graellsii*, *Parachondostroma miegii*, *Cobitis paludica*, *Aphanius iberus*, *Aphanius baeticus*, *Valencia hispanica*, among others (see Doadrio et al. 2011). As we have already said before, the geographical situation and its evolutionary history have turned the Iberian Peninsula into one of the regions with higher number of endemic freshwater fish species over the world and many of these species are critically endangered, endangered or vulnerable and represent some of the most endangered species on the planet (Doadrio et al. 2011; Maceda-Veiga 2012) (Figure 7).

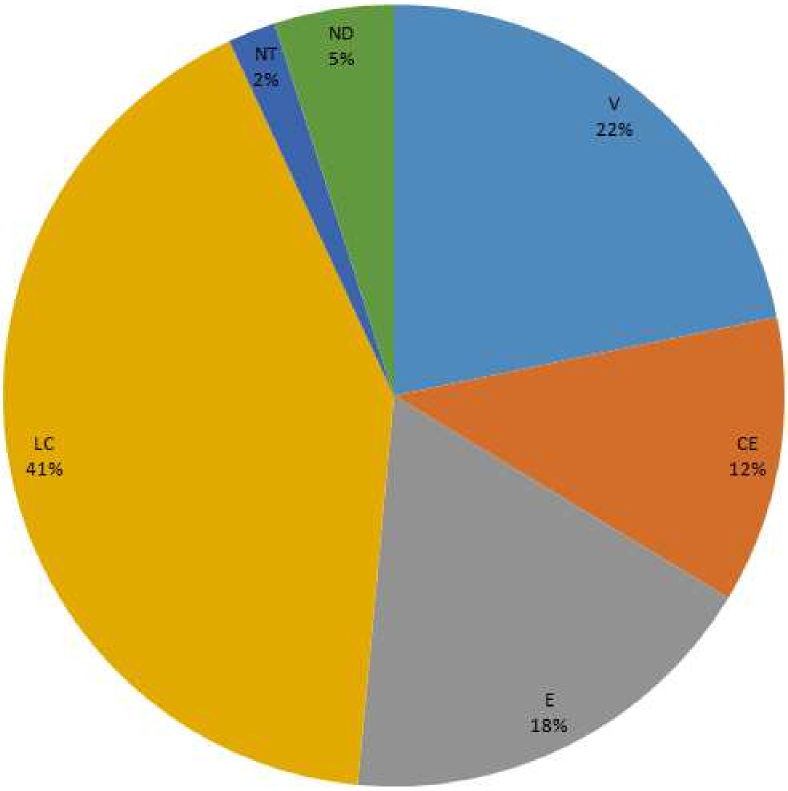


Figure 7. Current status of Iberian freshwater fish native species according to IUCN. CE) critically endangered, E) endangered, NT) near threatened, V) vulnerable, LC) least concern and ND) without enough data. This figure has been adapted from Maceda-Veiga (2012).

1.3.1 *Aphanius iberus* conservation and breeding programs in captivity

According to the International Union for the Conservation of Nature (IUCN), *A. iberus* (Iberian toothcarp) is one of the endangered species that are suffering a regression of its populations in the Iberian Peninsula due to the impact of the two main factors that affect native/endemic species such as destruction of its natural habitat and the introduction of invasive species (Crivelli 2006; Maceda-Veiga, 2012). Since 1990, there have been developed conservation programmes to preserve Iberian toothcarp and its gene pools (Araguas et al. 2007). Conservation programs for this species have been largely developed in the Autonomous Communities that host this species (Torralva et al. 2001). In general, breeding programs suppose an extended measure in fish conservation programs (Andrews and Kaufman 1994; Flagg et al. 1995; Berejikian 2000), and account for some successful reintroductions (Philippart 1995; Shute et al. 2005). However, many studies on fish rearing reveal that fish developed in captivity show notable differences in relation to their wild conspecifics and, in some cases, reintroductions of captivity rearing fish could cause ecological problems (Einum and Fleming 2001). For instance, some studies demonstrated that species reared in captivity showed divergent phenotypic development (Belk et al. 2008). In addition, studies with salmonids showed that captivity rearing diminished genetic diversity, and advanced the growth and the age of maturity (Horreo et al. 2017). This could be related to ecological differences in habitat conditions. The lack of natural selection in captive breeding might affect the population that is intended to be reintroduced. Normally, in natural conditions, the most vulnerable genotypes are eliminated by natural selection, whereas in captive breeding, this choice does not exist and therefore, these genotypes will persist and will be reintroduced into the natural habitat. This can cause differences regarding the behaviour, survival and growth of the reintroduced population, which could prevent the survival of these fish when released into the wild (Brown and Day 2002). Although the high plasticity in the life-history traits, morphology, metabolism, and diet among others, in relation to environmental variability is known in fish (Domenici et al. 2008; Killen et al. 2010; Almeida et al. 2012; Auer et al. 2015; Karjalainen et al. 2016), and that fish reared in captivity can alter their life-history traits related to adaptability (Le Cam et al. 2015), captive breeding programs of Iberian toothcarp have been carried out to help maintain its wild populations. As a consequence, it would be very important to have more information about the phenotypic plasticity of Iberian toothcarp under different environmental conditions, especially under captive breeding conditions, to ensure that the reintroduction of this species in natural habitats would be successful.

2. OBJECTIVES

The main objectives of this thesis are 1) to assess the phenotypic plasticity in life-history traits of an invasive species *Alburnus alburnus* in freshwaters of the Iberian Peninsula and 2) to evaluate the phenotypic plasticity in morphological and physiological traits of an endangered species *A. iberus* under wild and captive breeding conditions.

According to these objectives, the content of this thesis is structured in three chapters:

Chapter 4: Inter-population plasticity in growth, reproduction and dietary traits of invasive bleak *Alburnus alburnus* in the northeastern Iberian Peninsula

Chapter 5: Inter-population plasticity in growth, reproduction and dietary traits of invasive bleak *Alburnus alburnus* across the Iberian Peninsula compared to a native population of France

Chapter 6: Inter-population variability in physiology and morphology between wild and captive breeding populations of endangered species *Aphanius iberus*

3. MATERIALS & METHODS

3.1 Study areas

The study sites were situated at twelve locations distributed as follows (Figure 8): four small water courses in Catalonia (NE Iberian Peninsula), five in main rivers of the Iberian Peninsula and one in France regarding bleak (Chapters 4 and 5, respectively). In the case of Iberian toothcarp, two populations were analysed, one from the wild (Catalonia) and another one reared in captivity (Chapter 6) (see details below).

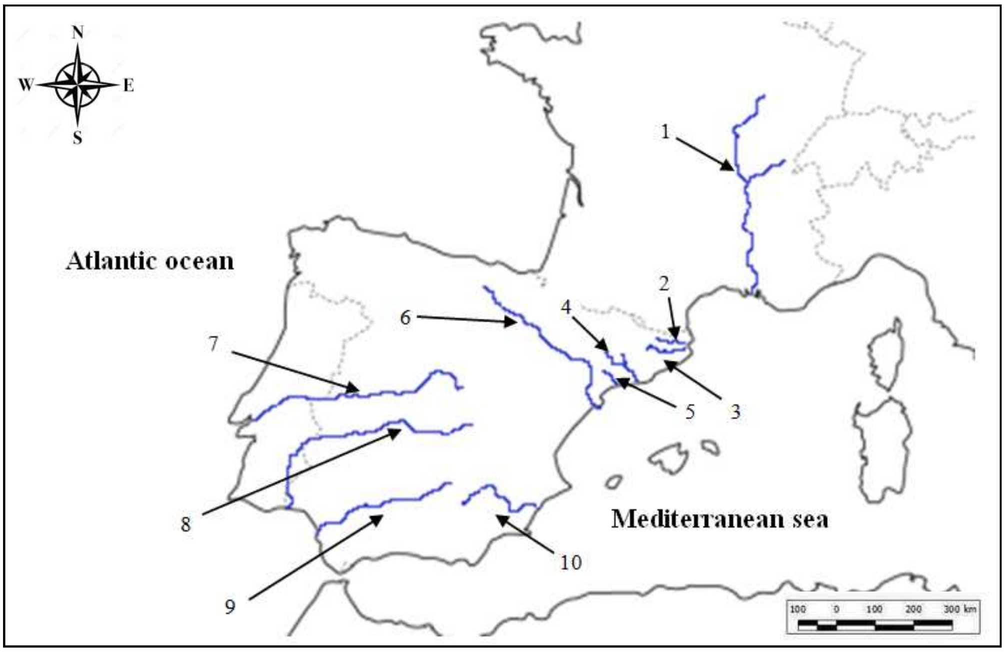


Figure 8. Rivers where *Alburnus alburnus* populations were surveyed for chapters 4 and 5. 1. Saône, 2. Muga, 3. Fluvià, 4. Cardener, 5. Foix, 6. Ebro, 7. Tagus, 8. Guadiana, 9. Guadalquivir and 10. Segura.

3.1.1 Catalonian small water courses (Chapter 4)

As regards to Chapter 4, we analysed four bleak populations that came from small water courses (i.e. streams, < 100 km river length), that are located at < 250 m a.s.l. in Catalonia (northeastern Iberian Peninsula) and drain into the Mediterranean Sea (from North to South): Muga (50º39’N/46º80’O), Fluvià (49º30’N/46º68’O), Cardener (39º95’N/46º24’O) and Foix (38º56’N/45º69’O) (see Figure 8). These streams were selected because they are geographically in close proximity to each other (latitude range 41°19′-42°16′ N, maximum distance ≈120 km) and, at the regional scale, possess similar fish assemblages, limnology and geomorphology, i.e. bed shape, wetted width, flow, substratum composition, riparian vegetation and level of human disturbances (see details in Catalan Water Agency 2015). Bleak abundances were also similar between study streams (CPUE = 7.6–9.6 ind 100 m^{-2}). All of these conditions allow that variations among bleak populations related to growth, reproductive and dietary traits, are more likely to be attributable to environmental factors operating at the local scale (i.e. within stream), such as food supply or habitat availability. Furthermore, the study streams show a typical Mediterranean hydrological regime (i.e. autumn-winter floods and summer droughts) and they can be used as reference systems for assessing the ecological responses of this invasive species when introduced to other freshwaters in the Mediterranean region of Europe (Almeida et al. 2014). A final key point for selection of these watercourses is that bleak was introduced in these four streams around the year 2000 and thus, their populations are currently well established and spreading in these habitats (Vinyoles et al. 2007). Consequently, the study populations are at the same ‘invasion stage’ and therefore, this potential effect on the assessed parameters was controlled.

3.1.2 Main rivers of the Iberian Peninsula (Chapter 5)

In Chapter 5, five main Iberian rivers were sampled, two sampling sites per river, along a latitudinal gradient (from North to South): Ebro (41º33’N/0º40’W), Tagus (39º54’N/4º30’W), Guadiana (38º56’N/6º03’W), Segura (38º00’N/1º12’W) and Guadalquivir (37º36’N–5º46’W) (see Figure 8). These geographic coordinates are the intermediate coordinates on the river, as two sampling sites were surveyed in each (see below). For comparative purposes, we surveyed a main river within the bleak’s native range close to the Iberian Peninsula, i.e. historically a potential ‘donor region’ for non-native fish introductions to this region along the so-called ‘Perpignan-Barcelona corridor’ (see Clavero and Garcia-Berthou 2006 for details on invasion routes). Thus, the River Saône (eastern France) was selected as a ‘reference’ population, although only one site was finally surveyed. In any case, a comparable number of bleak specimens (200 individuals, see below) were collected from the River Saône and data from the two sampling sites in Iberian rivers were pooled for statistical analyses (i.e. no ‘site effect’ was found, see Data Analyses below). The rivers Saône, Ebro and Segura drain into the Mediterranean Sea, whereas the rivers Tagus, Guadiana and Guadalquivir drain into the Atlantic Ocean. All the study rivers were selected because they represent large watercourses (i.e. >300 km river length), where bleak are widely distributed and reach relatively high abundances within the local fish assemblages. A final key point for selection of these particular Iberian rivers was that bleak were sequentially introduced into the different catchments and thus, their populations will potentially reflect particular ‘invasion stages’ on the examined growth, reproductive and dietary traits. Specifically, bleak were introduced into the study rivers in the following years (first mention) (Vinyoles et al. 2007): Ebro in 1992, Guadiana in 1999, Segura in 2004, Tagus in 2005 and Guadalquivir in 2006. The climate in the study area of the River

Saône is Temperate Oceanic (800–1000 mm of mean annual rainfall, 10–13 ºC mean annual temperature) (Ministry of Environment France 2019), the most common climatic conditions for the bleak's native range in Europe (Keith et al. 2011). The climate regime for the study Iberian rivers is typical Mediterranean, with rainfall concentrated in autumn-winter (≈500 mm) and intense summer drought (<100 mm). The mean annual temperature ranges between 15–18 ºC. The lowest temperatures occur in winter (down to –5 ºC) and the highest in summer (>35 ºC) (Ministry of Environment Spain 2019).

3.1.3 Wild and captive populations of *Aphanius iberus* from Catalonia (Chapter 6)

In Chapter 6, two populations of Iberian toothcarp were compared, one of them coming from a wild population that were sampled in the hypersaline coastal lagoon of Clot de la Llúdriga located in Aiguamolls de l'Empordà salt marshes (NE Iberian Peninsula) (Figure 9). The Aiguamolls de l'Empordà occupies the coast of the Bay of Roses, on the northern limit of the Iberian Mediterranean coast. It is a deltaic plain, originated by the contributions of the Muga and Fluvià rivers (Bach 1990) and consists of a set of coastal lagoons, Mediterranean marshes (not influenced by the tide) and flood areas, among which is the Clot de la Llúdriga where we can found wild populations of Iberian toothcarp (see Figure 12) (more details in Moreno-Amich et al. 1999). The other population studied comes from the same location but it was raised in captivity for a previous study in freshwater outdoor tanks (1.5 m × 1.5 m × 2 m) in the University of Girona, without any substrate or vegetation and under natural conditions of photoperiod, temperature and feeding.

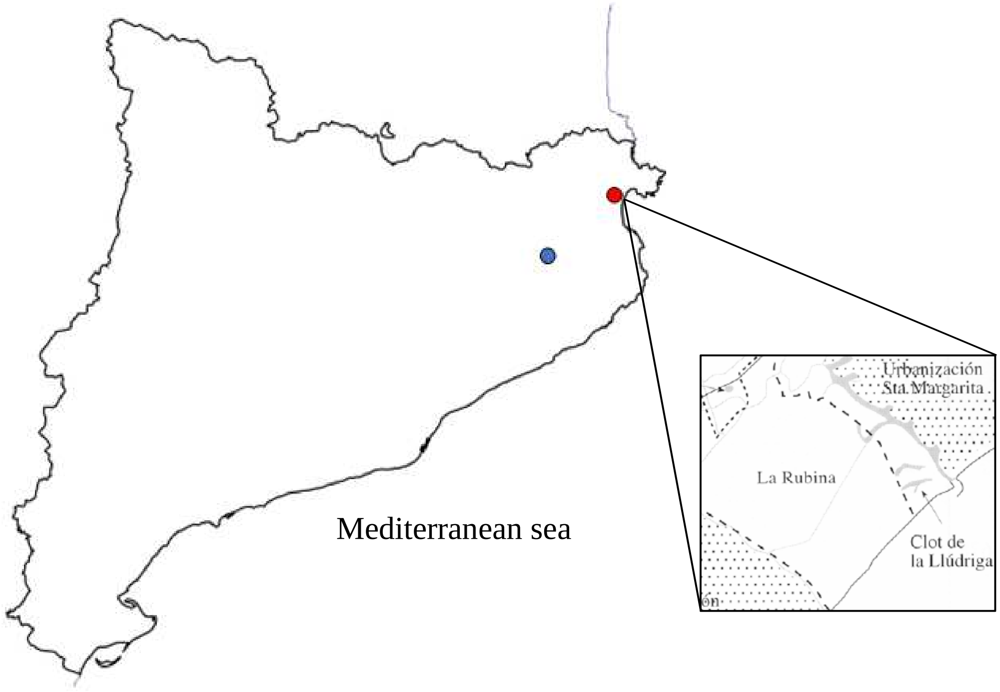


Figure 9. Sites where Iberian toothcarp populations proceeded for chapter 6. ● Indicates Costal lagoon Clot de la Llúdriga. ● Indicates University of Girona where captive population was reared. This figure has been adapted from Moreno-Amich et al. (1999).

3.2 Studied species

3.2.1 Bleak *Alburnus alburnus* (Linneaus, 1758)

In the Iberian Peninsula, bleak *Alburnus alburnus* (Linnaeus, 1758), is an exotic species that was introduced, legally and experimentally, at the beginning of the 20th century in Lake of Banyoles (Díaz-Luna and Gómez-Caruana 1998), although it was not established successfully. Bleak is a small fish of the cyprinids' family that can reach a maximum total length of 25 cm, although its average size is 15 cm (Billard 1997). Bleak can be found in fresh and brackish waters (salinities: 8–10 ‰) (Linden et al. 1979). It is a limnophile and bentho-pelagic species. It is native to central Europe, and its distribution covers from the northern slope of the Pyrenees to the Urals (Bogutskaya 1997). In Greece and the Italian and Balkan peninsulas it is replaced by a group of vicariant species closely related phylogenetically (Doadrio 2002). The bleak inhabits lentic (e.g. lakes, reservoirs) and lotic habitats (e.g. medium-large rivers). This fish species is highly tolerant to water pollution (Linden et al. 1979), being able to live in eutrophized lakes (Vinni et al. 2000), although it does not tolerate low oxygen waters (Willemsen 1980). The juveniles of small size inhabit the littoral zone of rivers and lakes. As they grow, they leave the shore and occupy more pelagic areas. Bleak is very abundant in large rivers and European lakes (Biró 1975; Wohlgemuth 1979) in which it presents a great variety of diet and use different types of habitats (Bíró and Muskó 1995). The temperature range of the water that this species can support is between 10 and 20 ºC (Baensch and Riehl 1991) although it is able to acclimate at temperatures of 37.7–40.6 ºC if the temperature increase occurs gradually (Horoszewicz 1973). In its native area, bleak feeds in open waters, mainly in lentic habitats, thus the diet is based on zooplankton (Herzig 1994; Vinni et al. 2000; Vasek and Kubecka 2004). The superior position of the mouth allows it to feed mainly on zooplankton (Vasek and Kubecka 2004), although other studies suggest that it also feeds on benthic invertebrates (Biró and Muskó 1995), insects and algae (Vøllestad 1985).

Figure 10. Bleak *Albunus alburnus*. This figure has been adapted from Lubomir Hlasek.

It is a species with a wide range of prey and it has the capacity to adapt its diet according to the availability of food offered by the habitat where it is found (Almeida et al. 2017). Variations have been described in bleak diet both in European (Chappaz et al.

1987) and Iberian reservoirs (Almeida et al. 2017). In European reservoirs and lakes, bleak can vary its position within the water column depending on the season of the year and depending on the circadian rhythms, feeding on zooplankton in the deepest areas during the day and rising towards the surface at night (where it feeds on terrestrial invertebrates that have fallen on the surface of the water) (Chappaz et al. 1987). Feeding increases from February to May and decreases in April, which coincides with the period of greatest activity in terms of reproduction (Politou et al. 1993). The length of sexual maturity in its native area ranges from 80 mm TL to 100 mm (males) or 120 mm TL (females) and reaches its sexual maturity at age 2–3 years (Froese and Pauly 2012). For this species to reproduce, the minimum temperature requirements are 14 °C (Souchon and Tissot 2012). The bleak has shown great variability in the parameters related to reproduction, such as fecundity (Mackay and Mann 1969), the diameter of eggs (Bonisławska et al. 2001) and energy investment in reproduction (Rinchard and Kestemont 1996). There have been documented changes in sex-ratio of this species that have been related to environmental factors. Fouzia and Abdeslem (2012) showed a higher proportion of females in the Keddara reservoir (Algeria) that was related to environmental factors such as oxygen and conductivity.

3.2.2 Iberian toothcarp *Aphanius iberus* (Cuvier and Valenciennes 1846)

Iberian toothcarp *Aphanius iberus* (Cuvier and Valenciennes, 1846) is a small endemic fish of the Aphaniidae family from the Iberian Peninsula, which is in danger of extinction (IUCN). This species presents sexual dimorphism. Males show vertical silvery and grey bands in the flanks that extend to the caudal fin (Figure 11). Females, which are larger than males, on their bodies present irregular dark spots (Figure 11) (Cuvier and Valenciennes, 1846).

Figure 11. Individuals of *Aphanius iberus* species. A and B) Male and female of *A. iberus*, respectively. This figure has been adapted from Ruiz-Navarro and Oliva-Paterna (2012).

This species inhabits coastal habitats, such as salt marshes, lagoons and some river mouths along the Mediterranean coast of the Iberian Peninsula. It is a bentho-pelagic species and its distribution is restricted to the Spanish Mediterranean coast (Doadrio 2002; Oliva-Paterna et al. 2006), from the Aiguamolls de l'Alt Empordà (Girona) to the Albufera de Adra (Almería) (Moreno-Amich et al. 1999; Gonzalez et al. 2017) (Figure 12).

Figure 12. Current distribution of *A. iberus* species. This figure has been adapted from Gonzalez et al. (2017).

This small fish (3-8 cm of length) is characterized by fast growth, early maturity, high reproductive effort and reduced life span (García-Berthou and Moreno-Amich 1992; García-Berthou and Moreno-Amich 1993). Iberian toothcarp is a euryhaline and eurythermal species, and it is adapted to fluctuating environments, where pronounced atmospheric events, such as storms or river floods can alter salinity in a matter of hours (García-Berthou and Moreno-Amich 1999).

Human impacts on its habitat (e.g. drying marshes and lagoons for agriculture, water pollution or aquifer overexploitation) and the introduction of invasive species, such as *Gambusia holbrooki*, have caused the reduction of its geographical distribution area, being limited its habitat to brackish and hypersaline waters in coastal lagoons and wetlands (García-Berthou and Moreno-Amich 1992; Doadrio 2001; Rincón 2002; Alcaraz et al. 2008). Nowadays, this species is listed on the Red List of Endangered Species of IUCN, being one of the few Iberian fish protected by national and international laws (Crivelli 2006). Furthermore, *A. iberus* is declared as "Endangered" in the Red List of Spanish Vertebrates (ICONA 1986) and in the Red Book of Vertebrates of Spain (Blanco and González 1992), as "Endangered of Extinction" in the

National Catalog of Endangered Species [RD 439/1990 (BOE 5.4.90)], as "Species of Protected Wildlife" in Annex III of the Berne Convention (1988), as "Species of general interest whose conservation requires the designation of special areas for their conservation" in the Annex II of the Directive of the Council of the European Union on the Conservation of Natural Habitats and of Wild Fauna and Flora (Directive 92/43 / EEC, Fauna-Flora-Habitats) and included in Annex II ("List of species in threatened or threatened ") of the Protocol on Specially Protected Areas and Biological Diversity in the Mediterranean (Barcelona, June 1995) (Torralva et al. 2001; Crivelli 2006). It is estimated that Iberian toothcarp has suffered a population decline of at least 50% in the last ten years (Fishbase 2019). Its distribution area is less than 500 km^2, it is fragmented and its populations are continually declining (Doadrio 2002; Fishbase 2019). Formerly, the populations of Iberian toothcarp showed a continuous distribution (Figure 12) but nowadays, fragmentation of its habitat has made that current populations remain isolated in the Mediterranean littoral area of the Iberian Peninsula (Doadrio et al. 1996; Oliva-Paterna et al. 2006; Alcaraz et al. 2008; Gonzalez et al. 2017). The causes of the decline of Iberian toothcarp populations are related to pollution, destruction of their habitats and the introduction of exotic species (Torralva et al. 2001; Crivelli 2006; Oliva-Paterna 2006; Araguas et al. 2007; Casas et al. 2011). Currently, there are no more than twenty wild populations of Iberian toothcarp and some of these populations are constantly decreasing in abundance (Oliva-Paterna et al. 2006).

3.3 Field sampling

Chapters 4 and 5 focused on non-native species *A. alburnus* in the Iberian Peninsula. All fish sampled were collected from May to June 2012, just before the spawning period of bleak for each study area, and thereby avoiding any effect of the “reproductive stage” on diet. Moreover, year 2012 is considered to have been hydrologically ‘average’ in the study areas (Ministry of Environment France 2019; Ministry of Environment Spain 2019). As a result, the effects of particular dry or wet years on growth, reproductive and dietary traits are avoided within our studies, which allows the data to be considered representative for this species in the invaded Mediterranean region of Europe.

In Catalonian small waters courses (Chapter 4), bleak were collected along 10 km river (*n* = 10 sampling sites, one site per km) in the middle reach of each stream by electrofishing (2000 W pulse DC generator at 200-250 V, 2-3 A) and dip nets (1.5 m long pole, 30 cm diameter net, 10 mm mesh size). The sampling method consisted of following a zigzagging and upstream direction at each site (50 m river long, 30 min). To encompass the existing environmental variability, fish were collected from all meso-habitats present in the study streams (i.e. runs, riffles and pools), thus obtaining a representative sample of bleak across the broadest possible body size range from every stream. Finally, bleak individuals (*n* = 336: 84, 88, 83 and 81 from Muga, Fluvià, Cardener and Foix streams, respectively) were stored in ice during transport to the laboratory.

Sites sampled in main rivers of the Iberian Peninsula (Chapter 5) were 11 (2 per Iberian river plus 1 in the River Saône). To encompass similar environmental conditions, sites were specifically located in well-regulated middle reaches of the main channel, where water level fluctuations are controlled throughout the year, avoiding the effects of strong increases/decreases in river discharge. These habitat conditions are representative of large rivers in both the native and Iberian ranges. In addition, sampling sites were located near similar surroundings (e.g. land use for agricultural exploitation) and far from influences of main tributaries and towns. Finally, sites were separated >50 km within each Iberian river to ensure the data were more representative from the study areas and also to minimise data dependence among sampling sites per river. A variety of consistent sampling protocols was followed by wading and from boats according to the European legislation (CEN/ISO Standards, EC Directive 2014/101/UE 2014). This allowed obtaining a representative sample of bleak across the broadest possible body size range from each river. Catch methods consisted of following a zigzagging and upstream direction in both banks at each site (100 m river length) by electrofishing (2000 W pulse DC generator at 200–250 V, 2–3 A, 30 min per bank), dip nets (1.5 m long pole, 30 cm diameter net, 10 mm mesh size), seine nets (20 × 2 m, 10 mm mesh size) and gill nets (20 × 1 m, 10 mm mesh size, 50% hanging ratio, 1.5 m deep). All surveys followed the same sampling protocols (e.g. proportional effort in terms of people and time) to ensure comparability among the study rivers. To encompass the existing environmental variability, fish were collected from all meso-habitats present in the study rivers (e.g. runs, pools, shallows). Bleak individuals sampled in this study (n = 1200, 100 ind. × 2 sites × 5 Iberian rivers + 200 ind. × 1 site from the River Saône) were stored in ice during transport to the laboratory.

Regarding to Chapter 6, specimens of wild population (n = 30) were collected in July 2017 using a dip net from the hypersaline coastal lagoon of Clot de la Llúdriga from Aiguamolls de l’Empordà salt marshes (NE Iberian Peninsula). Captive population that comes from a previous study has been maintained in captivity in freshwater tanks under semi controlled conditions during 5 years. It was reproduced naturally, there were no predators nor other fish species in the tanks and it was fed on the resources that produced the same tank. Both, wild and captive populations were transported to laboratory in tanks with supplied oxygen (two battery-operated aerators with portable pumps).

3.4 Laboratory procedures

When all individuals of the non-native bleak *A. alburnus* arrived at the laboratory, were measured for total length (TL, ± 1 mm). Also we measured standard length (SL, ± 1 mm) to avoid ‘noise’ given by variation of caudal fin length not related to body size (e.g. wounds and cuts in the fish skin and rays). Bleak were dissected to examine the sex. Eviscerated weight (eW, ± 0.1 g) was recorded to avoid the effect of gonad and gut masses. Regarding to reproductive parameters (except for sex-ratio), traits were analysed in mature females only, as this “fraction” of fish population is the most relevant for the assessment of future viability and subsequent invasiveness in small Mediterranean-type rivers (Vila-Gispert et al. 2005). Therefore, mature females were also measured for gonad weight (Wg, ± 0.1 mg) by using an electronic balance. Sub-samples (0.01 g) were taken from anterior, middle and posterior portions of each mature ovarian lobe. Yolked oocytes were counted in every sub-sample and measured for egg diameter (ED, ±0.01 mm) by using a PC-based image analysis (Aphelion, ADCIS, Saint-Contest, France). The age of the fish was determined by counting true annuli from acetate impressions of scales and read on micro projector (magnification: 48×). Age determinations were completed independently by two readers and when the interpretations were different, an additional reading was made. If the disagreement continued, then the sample was excluded from the analyses. The total scale radius and radius of annual increments were measured from the focus to the posterior edge along the anterior-posterior axis. Regarding to diet analyses, only the previous one-third of the intestinal tract was preserved in 4% formalin for subsequent examination to avoid any severely digested food remains. Food items were identified to the lowest possible taxonomic level (e.g. Tachet et al. 2002) using a dissecting microscope (40×) and weighed using an electronic balance (wet weight to within 0.1 mg).

As regards to Iberian toothcarp study, prior to the swimming performance trials, captive and wild fish were acclimated to experimental conditions for 2 weeks. Fish were placed into glass aquariums (90 l) at a temperature of 25 ± 1°C under a natural photoperiod cycle. At least 90% of the air saturation in aquariums was provided by vigorous aeration. The fish were fed with frozen bloodworms *Chironomus* sp. once a day until satiety from the second day in the aquariums. There was no mortality during the acclimation period. To avoid postprandial effects during the experiment, feeding was interrupted for 24 h before the experiment (Fu et al. 2009). After the swimming performance tests, fish were quarantined for a week before being returned to their natural habitat.

3.4.1 **Swim tunnel set up**

To determine critical swimming speed and metabolism for the wild and captivity-reared populations of Iberian toothcarp, we used the methodology summarized as follows: respirometry was conducted using a mini swim tunnel (Blazka-type design) with a non-turbulent laminar flow and equipped with a continuous-flow respirometer (Loligo® Systems, Viborg, Denmark) (Figure 13). A propeller connected to the motor outside of the respirometer generated the flow. The circular test section (170 ml volume, 100 mm length × 26.4 mm internal diameter) was laterally covered with non-reflecting white screens to avoid disturbing fish by ‘mirror effects’ (Srean et al. 2017b). An external buffer tank was connected to the respirometer and an automated flush pump flushed the air-saturated water inside the swim tunnel to ensure well-mixed water in the respirometer. The temperature in the swim tunnel was kept constant at 24–25 °C by using a heater (Eheim Jäger Model 3613; EHEIM GmbH and Co KG, Deizisau, Germany). To assess critical swimming speed fish were placed into the respirometer and allowed to acclimatize to an initial velocity of ca. 0.5 $BL \cdot s^{-1}$ (body length, taken as the standard length of the fish, per second) for 2 h to recover from handling stress. After that, velocity was progressively increased ca. 1 $BL \cdot s^{-1}$ every 20 min, which is enough to detect changes in dissolved oxygen concentration (Plaut 2001). During the flush phase, oxygen concentration inside the tunnel was supplied from the buffer tank up to 100% saturation for approximately 2-min period (Rosewarne et al. 2016). Oxygen levels never dropped below 70% of saturation to prevent hypoxia-related stress (Blaikie and Kerr 1996). Measurements of oxygen consumption were recorded using an optical fibre oxygen instrument (Witrox 1; Loligo® Systems, Tjele, Denmark). For calibration purposes, two-point calibration with the oxygen sensor was used to record the highest concentration value as 100% air-saturated and the lowest concentration value as 0% using a solution of sodium sulphite (Na_2SO_3, 0.159 M). Background microbial respiration inside the respirometer was calculated with no fish for 10 min at the end of each trial and was used as ‘blank’.

Figure 13. Respirometer (1.5 l) used for measurements of critical swimming capacity and metabolism. Image extracted from http://www.loligosystems.com.

3.5 Data and statistical analyses

To determine TL at ages of *A. alburnus* individuals of all populations studied, non-linear and linear equations were fitted to determine which model best described the relationship between TL and scale radius. As the resulting models were linear equations for the six bleak samples, back-calculation of TLs at age were estimated by the Fraser–Lee equation (adapted from Francis 1990), as follows:

$$\mathrm{TL}_A = \mathrm{TL}_0 + (\mathrm{TL}_C - \mathrm{TL}_0) \times (R_A : R_C)$$

where TL_A is TL when growth mark (i.e. *annulus*) *A* was formed, TL_C is TL at the time of capture, R_A is the distance (i.e. scale radius) from centre to the Annulus *A*, R_C is the total scale radius at the time of capture and TL_0 is the intercept on the length axis from linear regression between all TL_C and R_C values. Thus, the overall intercept TL_0 acts as a ‘weighting factor’ to reduce bias resulting from differences in the size distribution of the examined populations see (Tarkan et al. 2016 for the same procedure). The particular TL_0 value was 14mm, which was used as a fixed TL–scale intercept.

For comparisons of growth rates, the Hickley and Dexter (1979) procedure was followed by using back-calculated data, as follows: (1) mean TLs from all populations were calculated at every age A, which were plotted against each mean TL at age $A + 1$ to obtain a regression line for the Walford (1946) method (used below); (2) expected TLs at every age (eTL_A) were obtained from the formulae (adapted from the von Bertalanffy growth equation):

$$\mathrm{eTL}_A = \mathrm{TL}_\infty \times (1 - S^A) \quad \text{and} \quad \mathrm{TL}_\infty = \mathrm{TL}_Y \div (1 - S)$$

where TL_Y and S are the Y-intercept and the slope of the Walford plot respectively; (3) all back-calculated TLs at every age A were expressed as a percentage of each eTL_A; and (4) the mean percentage from different ages was used to calculate a growth index (GI, %) for each bleak individual. ANOVAs were used to test for significant differences of back-calculated TLs and GI between populations. To provide an integrated quantification of body condition (BC), eW was used to avoid bias from gonad mass and gut content. To assess reproductive investment (RI) in mature females, gW was used (Masó et al. 2016). Female fecundity (FEC) was estimated according to the formula

$$\mathrm{FEC} = \mathrm{gW} \times D$$

where FEC is the number of yolked oocytes in the entire ovary, gW is the gonad (ovary) mass and D is the density of yolked oocytes (number of oocytes g^{-1} of ovarian tissue). ANCOVAs were used to test for significant differences in BC (covariate: TL), RI, FEC and ED (covariate: eW).

Mean age-at-maturity (AaM) of each population was calculated from the percentage of mature females in each age class, using the following formula (adapted by Fox and Crivelli 2001):

$$AaM = \sum^{A_{\max}} A\left[M_A - M_{(A-1)}\right]$$

where A is the age in years, M_A is the proportion (from 0 to 1) of mature fish at Age A, and $A_{\max}$ is the maximum age in the fish sample. A modified version of this formula (10-mm-TL intervals in place of age classes) was used to calculate mean TL at maturity (LaM), as per Fox and Crivelli (2001).

GLMs were used to test for differences between males and females on the examined growth traits (i.e. back-calculated TLs, GI and BC), as per Almeida et al. (2014). Because no difference was found, this categorical factor (i.e. sex) was not included in subsequent models, so as to increase the statistical power of the remaining sources of variation (see the same procedure in (Alcaraz and García-Berthou 2007). Data were pooled per Iberian river because the effect of 'sampling site' within each river in previous GLMMs was not significant (using 'site' as the random factor) a comprehensive review of this statistical technique is given in Johnson et al. (2015). ANOVAs and ANCOVAs were followed by *post hoc* Tukey–Kramer honestly significant difference (HSD) tests. Sex ratio (SR, male:female) was compared among rivers using pairwise Chi-Square (x^2) tests (with Yates' correction). Data were transformed into $\log_{10}(x + 1)$ except percentage data, which were logit-transformed (Warton and Hui 2011). Assumptions of normality of distributions and homogeneity of variances were verified through Shapiro–Wilks and Levene's tests respectively.

Regarding to dietary traits of all *A. alburnus* studied in this thesis, percentage of occurrence (omitting empty guts) and percentage of ingested mass (relative to the total ingested mass in all examined fractions of intestinal tracts) were calculated for each food category. Four dietary parameters were calculated for each fish: ingested mass (mg), prey richness (*S*), trophic diversity (Shannon index, *H'*) and trophic niche breadth (Levin index, *B*). Fulton's condition factor (*K*) was also calculated. Preliminary analyses found no difference between sexes, thus this categorical factor was not included in subsequent data analyses. ANCOVA was used to reveal significant differences between populations for the four dietary parameters. The effect of body size was tested by using total length (TL) as the covariate, as this was a better predictor for dietary parameters than either eW or *K*. Ancovas were followed by a *post hoc* Tukey-Kramer honestly significant difference (HSD) test. Data were transformed by using ln (x + 1). Data normality of distributions and homogeneity of variances were verified through Shapiro-Wilks and Levene's tests, respectively. All statistical analyses were performed with SPSS v.19 (SYSTAT Software Inc., Chicago, U.S.A.). The significance level was set at $\alpha = 0.05$. Sequential Bonferroni corrections were performed for every set of multiple tests.

Related to the Iberian toothcarp study, critical swimming speed (U_{crit}, cm s^{-1}) was calculated following (Brett 1964):

$$U_{\mathrm{crit}} = U_f + [U_{\mathrm{i}}(\mathrm{T}_{\mathrm{f}}/\mathrm{T}_{\mathrm{i}})]$$

where U_f is the highest velocity maintained for a full 20-min period (cm s^{-1}), T_f is the time swum at the last velocity increment (min), T_i is the interval time set (20 min in this case), and U_i is the velocity increment (cm s^{-1}). The experiment finished when fish were exhausted and could no longer swim against the current (Farrell 2008). Swimming speeds were not corrected by the ‘solid-blocking effect’ because the cross-sectional area of the fish never overcame 10% of that of the respirometer (Bell and Terhune 1970).

Oxygen consumption was calculated by fitting linear regression of the oxygen concentration decline over time at each velocity. The resulting slope or regression coefficient was used to calculate oxygen consumption rates (MO_2, mg O_2 h^{-1}):

$$MO_2 = -(\Delta Of - \Delta Ob) \times V$$

where ΔOf and ΔOb are the rates of oxygen consumption in mg O_2 l^{-1} min^{-1} due to fish respiration and microbial respiration, respectively, and V is the volume of the respirometer. Afterwards, oxygen consumption rates were then recalculated in mg O_2 h^{-1}. Maxium metabolic rate (MMR) was determined as the highest value of MO_2 during swimming trial, which was usually close to the fastest velocity (Srean et al. 2017). The exponential function was used to describe relationship between MO_2 and swimming speed (Brett 1964; Webb 1975; Beamish 1978):

$$MO_2 = \mathrm{SMR} \times e^{cU}$$

where SMR, the standard metabolic rate; c, is a constant and U is the swimming speed. SMR was determined by extrapolating to zero activity (Brett 1964). The absolute aerobic scope (AAS) was calculated as the difference between MMR and SMR (Clark et al. 2013). After the experimental procedure, each individual was measured (standard body length, SL) to the nearest 1 mm and weighed (fish mass, M) to the nearest 0.1 mg.

To test for significant variations in critical swimming capacity and metabolism between the two populations of Iberian toothcarp, we conducted an analysis of the covariance (ANCOVA) in which population (wild *versus* captive) and sex (male and female) and the interaction between population and sex served as fixed effects. Critical swimming speed, SMR, MMR and AAS were used as dependent variables, and standard length (SL) and fish mass (M) as the covariates. ANCOVA R-square for critical swimming speed had higher values with standard length as a covariate than fish mass (M), whereas ANCOVA R-squares for SMR, MMR and AAS were the highest with fish mass (M) as a covariate. Thus, for results and further analyses of critical swimming speed, we used SL and for metabolic traits (SMR, MMR and AAS) we used M. The assumption of parallelism of ANCOVA was tested analysing the interactions between the covariate and the categorical factors. If such interactions are significant, they indicate that the slopes are not homogeneous and the assumption of parallelism is thus not satisfied. When interactions were found to be non-significant, they were removed from the model in order to improve the statistical power of the ANCOVA (García-Berthou and Moreno-Amich 1993). If the interaction between species and sex was found significant, an ANCOVA was separately performed for each population to test for differences between sexes. Statistical analyses were conducted with the SPSS Statistics 25.

3.5.1 Morphometric analysis

Individuals of Iberian toothcarp that performed well in the swim tunnel and successfully completed the test, were analysed using geometric morphometry, which is based on the modifications of the coordinates of the "landmarks" and their covariance, in order to determine if morphological differences between populations and sex could be related to the critical swimming speed and metabolism.

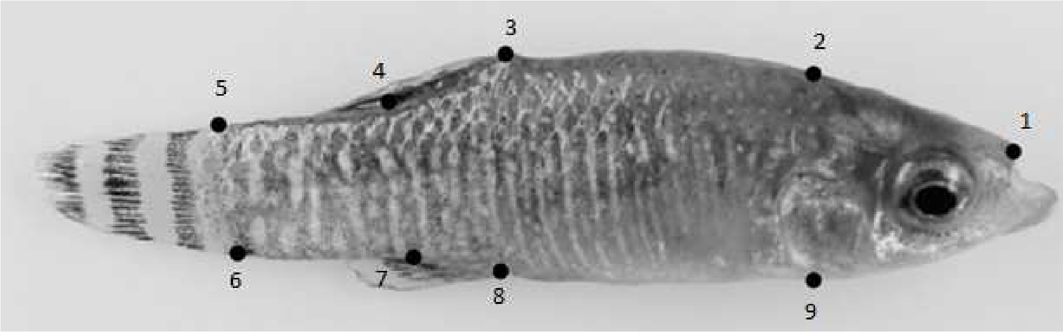


Figure 14. Location of the 9 landmarks used in the geometric morphometric analysis to characterize the body shape variation between the two Iberian toothcarp populations. 1) Tip of the snout; 2) Dorsal head end; 3) Anterior dorsal fin insertion; 4) Posterior dorsal fin insertion; 5) Dorsal caudal fin insertion; 6) Ventral caudal fin insertion; 7) Posterior anal fin insertion; 8) Anterior anal fin insertion; 9) Beginning of the operculum.

Digital photographs were taken with a Canon Handycam DS126491 (Japan) (image resolution of 18 Mp) of the right side of each specimen, and 9 landmarks were defined and recorded as two-dimensional (*x* and *y*) coordinates (Fig. 14) using the software ImageJ 1.50i (Schneider et al. 2012). Landmark coordinates were adjusted with a generalized Procrustes analysis (GPA; Rohlf and Slice 1990). This procedure centres each specimen onto a common centroid, scales all specimens to a common unit size by dividing each total configuration by centroid size and lastly rotates each specimen to a common orientation that minimizes the differences between corresponding landmarks. We estimated a ‘consensus’ form composed of the mean coordinates for each landmark averaged across all specimens. For each specimen, we estimated 18 partial warps plus the corresponding centroid size using the MorphoJ 1.06d program (Klingenberg 2011). To help visualising and interpreting shape differences we used deformation grid plot which reflect the degree and type of shape change between the consensus form and the form of each group (population by sex combinations) analysed.

Partial warps are the minimal shape parameters needed to deform the ‘consensus’ configuration to each one of the analysed specimens and contain shape information which will be analysed using multivariate statistics (MANOVA). Next, we conducted a multivariate discriminant function analysis (DFA, Wilks’s method) on partial warps matrix to highlight shape differences between populations and sexes of Iberian toothcarp. This analysis included a cross-validation procedure to identify the percentage of correct classifications by comparing the morphology-related classification of each specimen made by the DFA with our *a priori* classification of each specimen into one of the population by sex group. Individual scores associated with the first two canonical axes of the DFA were correlated (Pearson’s correlation) with individual critical swimming speed and metabolism (SMR, MMR and AAS) to assess the relationship between morphological variables and fish swimming performance. Statistical analyses were performed with the SPSS Statistics 25.

RESULTS & DISCUSSION

4. Inter-population plasticity in growth, reproduction and dietary traits of invasive bleak *Alburnus alburnus* in northeastern Iberian Peninsula

4.1 Abstract

The bleak *A. alburnus* is an invasive fish in the Iberian Peninsula, where this cyprinid species disturbs the highly endemic fish fauna. To provide insights into bleak autoecology, the aim of this study was to compare growth, reproduction and dietary traits across four Iberian streams (northeastern Spain): Muga, Fluvià, Cardener and Foix. These streams have similar environmental conditions at the regional scale (e.g. Mediterranean climate, geomorphology), which allow that variations among bleak populations are more likely to be attributable to environmental factors operating at the local scale (i.e. within stream). Bleak were collected in May–June 2012, just before the spawning period. In Cardener stream, bleak showed high back-calculated lengths at ages 1 and 2, growth rate, body condition and reproductive investment. In Foix stream, bleak showed low back-calculated length at age 2, growth rate, body condition and proportion of females, whereas length at maturity reached the highest value. Regarding dietary traits, Diptera larvae were the most common prey in percentage of occurrence. As percentage of ingested mass, flying insects (e.g. just emerged Ephemeroptera) was the most important food category in Muga and Fluvià streams, other benthic invertebrates (e.g. Gastropoda) in Cardener stream and Diptera larvae in Foix stream. Remarkably, Foix population showed the highest intake of plant material and the lowest values for total ingested mass, prey richness, trophic diversity and trophic niche breadth. Overall, results indicate that population 'status' appears to be better in Cardener stream, whereas in Foix stream, environmental conditions may be poorer for bleak. Present findings suggest that the wide inter-population plasticity displayed by bleak allows this non-native fish to more successfully invade Mediterranean-type streams in the Iberian Peninsula. This better understanding of bleak population traits

may facilitate the prediction of the most likely areas to be colonized by this invasive species throughout Iberian freshwaters.

4.2 Results

Bleak individuals ranged from 43 to 144 mm TL, and bleak mass from 0.7 to 45.3 g eW. Significant growth differences were detected among back-calculated TLs at age 1 in the four examined populations ($F_{3,262} = 36.06$; $P < 0.001$), with bleak from Cardener stream showing the highest mean back calculated TL and Muga population showing the lowest mean TL. Bleak populations in Foix and Fluvià streams made a different significant group for TL at age 1 (Table 4.1). Differences were also found for the back-calculated TL at age 2 ($F_{3,101} = 21.66$; $P < 0.001$), with Cardener and Fluvià populations showing the greatest mean TLs, whereas Muga and Foix populations showed the lowest mean values (Table 4.1). Age 3 was only found in Foix and Fluvià populations, with the latter stream showing the highest TL (Table 4.1). Significant differences were found between populations for the GI ($F_{3,317} = 4.83$; $P < 0.01$), which showed that the fastest bleak growth was in Cardener and Fluvià streams, and then Foix and Muga populations (Table 4.1). The highest and the lowest BC were observed in Cardener and Foix/Fluvià streams, respectively, with significant differences between populations ($F_{3,316} = 70.08$; $P < 0.001$). Bleak from Muga stream made a third group intermediate between Cardener and Foix/Fluvià streams (Table 4.1). Regarding reproductive parameters, significant differences were found between populations for RI ($F_{3,93} = 3.25$; $P < 0.05$), with the highest adjusted Wg value in Cardener population, followed by a second group made by Foix, Muga and Fluvià streams (Table 4.1). The highest mean LaM was found in Foix stream, then Cardener and both Muga and Fluvià populations showed the lowest values (< 80 mm LaM) (Table 4.1). The youngest mean AaM was found in Fluvià stream, whereas the oldest mean for this parameter was found in Cardener stream, with both Foix and Muga populations showing intermediate values (Table 4.1). All bleak populations were dominated by males, with the Muga population possessing the highest sex-ratio ($\chi^2_3 = 28.94$; $P < 0.05$), followed by Foix, Cardener and Fluvià streams (Table 4.1).

Regarding dietary traits, diptera larvae and flying insects (e.g. just emerged Ephemeroptera) were the most common prey in the studied populations (Table 4.2). In terms of ingested mass, flying insects were more important in the Muga and Fluvià streams. In the Cardener stream, other benthic invertebrates (e.g. Coleoptera larvae or Gastropoda) and Diptera larvae reached the greatest percentages of ingested mass. Diptera larvae and plant material were the most important food mass in the Foix stream (Table 4.2). Significant differences were found among the bleak populations in terms of ingested mass ($F_{3,321} = 4.78$; $P < .01$), prey richness ($F_{3,321} = 35.46$; $P < .001$), trophic diversity ($F_{3,321} = 32.46$; $P < .001$) and trophic niche breadth ($F_{3,321} = 25.69$; $P < .001$). Specifically, the least value of ingested mass was found in the Foix stream (<10 mg, Fig. 4.1). The remaining parameters had the same pattern, with bleak from the Muga stream reaching the highest mean, followed by the Fluvià and Cardener streams, and then the Foix population (Fig. 4.1).

Table 4.1. Populations traits in growth and reproduction of bleak *Alburnus alburnus* for every study stream: back-calculated total length (TL, mm) at ages (An), Growth index (GI, %), body condition (BC, We in g), reproductive investment (RI, Wg in g), TL at maturity (LaM, mm), age at maturity (AaM, years) and sex-ratio (male ÷ female). Results are means (An, GI)/adjusted means (BC, RI) ± SE. Significant differences between bleak populations are shown by superscripts, after Tukey HSD and χ^2 test ($P < 0.05$).

Study stream	A_1	A_2	A_3	GI	BC	RI	LaM	AaM	Sex-ratio
Muga	^{a}47.9 ± 5.7	^{a}86.2 ± 5.7	-	^{a}86.2 ± 6.5	^{a}8.75 ± 0.24	^{a}1.29 ± 0.24	79.8	0.91	^{a}2.86
Fluvià	^{b}58.1 ± 5.6	^{b}98.5 ± 4.1	125.9 ± 6.4	^{b}106.9 ± 6.7	^{b}7.76 ± 0.22	^{a}1.11 ± 0.23	79.1	0.86	^{b}1.36
Cardener	^{c}75.0 ± 2.5	^{b}100.3 ± 4.7	-	^{b}116.1 ± 6.4	^{c}11.02 ± 0.19	^{b}2.33 ± 0.20	87.0	1.14	^{b}1.64
Foix	^{b}60.3 ± 6.1	^{a}79.3 ± 7.2	[1]86.8	^{a}88.9 ± 5.0	^{b}7.43 ± 0.20	^{a}1.44 ± 0.21	92.0	1.00	^{b}1.97

[1] Only one A_3 specimen was collected in Foix Stream

Table 4.2. Diet composition of bleak *Alburnus alburnus* from Muga, Cardener, Fluvià and Foix streams. Percentages of occurrences (Oc., %) and ingested masses (Mass, %) are shown.

Stream	Muga		Cardener		Fluvià		Foix	
Food category	Oc.	Mass	Oc.	Mass	Oc.	Mass	Oc.	Mass
Algae and plant debris	54	11	18	2	17	10	4	25
Zooplankton	5	1	6	<1	25	4	5	1
Ephemeroptera and Plecoptera nymphs	20	2	18	1	6	4	2	3
Odonata nymphs	20	5	3	<1	1	1	1	<1
Diptera larvae	93	34	94	22	61	16	87	33
Trichoptera larvae	27	2	5	<1	2	4	9	19
Other benthic invertebrates	12	3	12	57	10	1	4	1
Nektonic insects	20	2	2	<1	10	1	9	<1
Flying insects	88	40	24	17	45	59	19	17

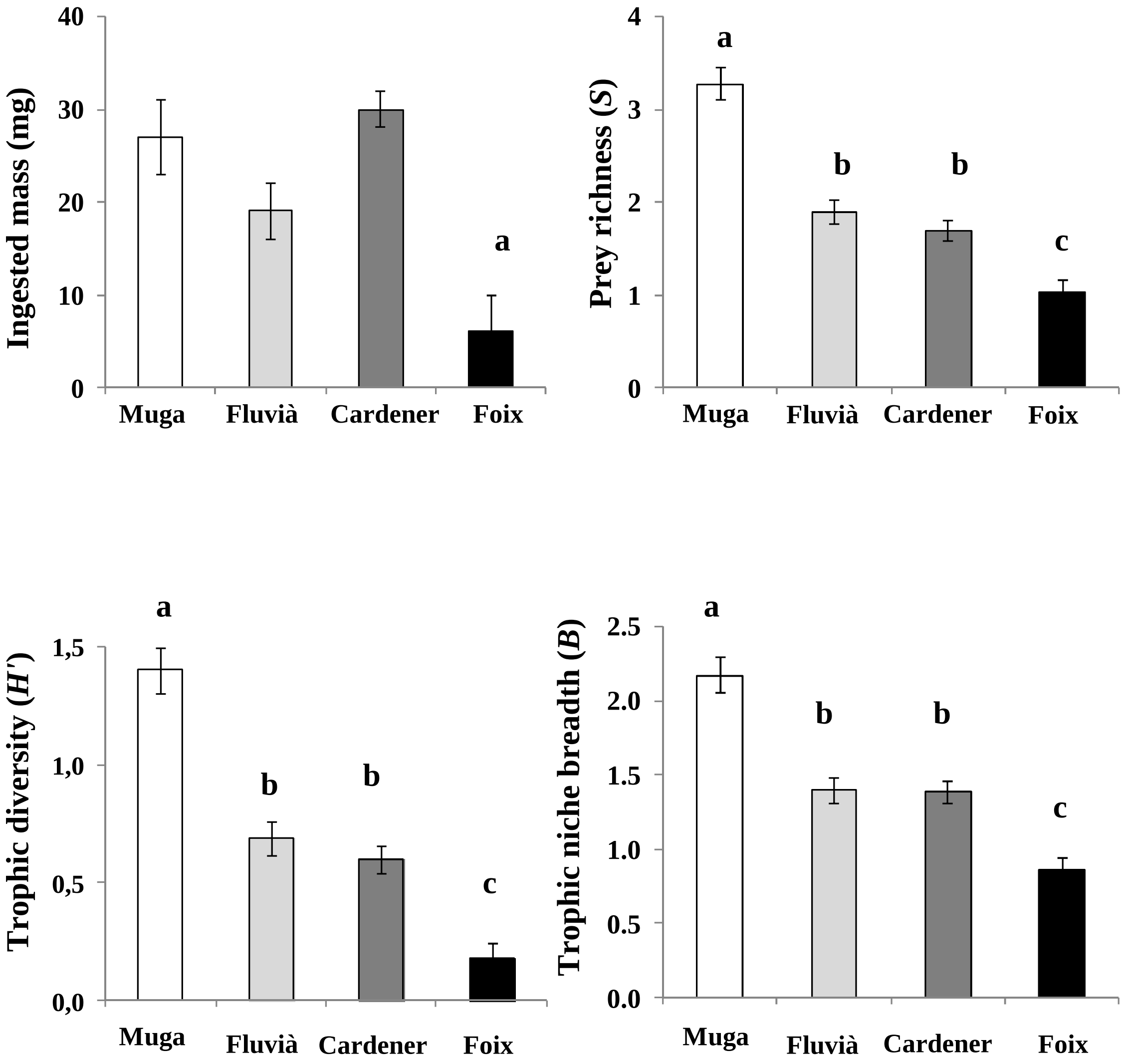


Figure 4.1. Comparison of four dietary parameters for bleak *Alburnus alburnus* among studied streams. Results are adjusted means ± SE, after ANCOVA (covariate: TL). Sample size (*n*) = 326. Sampling period: May–June 2012. Letters above columns indicate significant differences in bleak populations, after Tukey HSD tests ($P < 0.05$).

4.3 Discussion

Life-history traits are expected to change in animal populations in response to variations in biotic and abiotic conditions at the local scale (Stearns and Koella 1986), with this being particularly clear in freshwater fishes (Fox and Crivelli 2001). These changes also apply to non-native species when invading new habitats (e.g. Tarkan et al. 2012), where they usually display high phenotypic plasticity and thus contribute to a more successful invasion process (Agrawal 2001). In particular, Mediterranean-type rivers typically show a high hydrological variability, including autumn-winter floods and summer droughts, which may promote great differences in local conditions between contiguous catchments (Boix et al. 2010). Accordingly, the population parameters examined in the present study were highly variable between the study streams, suggesting wide phenotypic plasticity in bleak. Specifically, bleak population “health” appears to be better in Cardener stream, where growth, body condition and reproduction showed the fastest rate, best status and highest investment, respectively. On the contrary, bleak populations showed overall lower growth rate, body condition, reproductive investment and proportion of females in Muga and Foix streams. The ability of this invasive fish to overcome changes in local conditions has been also shown elsewhere in the Iberian Peninsula by Almeida et al. (2014), regarding size structure between contrasting habitats (river *versus* reservoir). Beside this, the present findings on growth and reproduction also suggest that the wide interpopulation plasticity displayed by non-native bleak may be a mechanism for this species more successfully to invade novel Mediterranean freshwater ecosystems. The observed LaM and AaM in the study streams were lower than in native populations under similar climate conditions (e.g. > 100 mm TL and ≈2 years, see Politou 1993). These two traits (i.e. low length and age at maturity) are typical in populations that are in an expansion stage, facilitating the subsequent bioinvasion (Bohn et al. 2004). Studies with a variety of fish species, including non-native populations, have demonstrated that patterns of body development vary in relation to abiotic and biotic factors operating at the local scale such as temperature, competition or food availability (e.g. Tarkan et al. 2010, 2012). These findings also support the hypothesis that bleak could change their growth and reproductive traits under particular conditions within every study habitat (Almeida et al. 2014). Thus, non-native species may switch their life-history strategies at the initial stage of invasion when they are introduced in novel ecosystems and confronted with variable environmental scenarios (Ribeiro and Collares-Pereira 2010; Tarkan et al. 2012). This might be related to epigenetic mechanisms in which some progeny of invaders show a better survival rate than those of species in its native range. Such phenotypic plasticity has been shown for several non-native fish species belonging to a variety of taxonòmic families: Gobiidae such as bighead goby *Ponticola kessleri* and round goby *Neogobius melanostomus* (L'avrinčíková and Kováč 2007, Kováč et al. 2009), Cyprinidae such as topmouth gudgeon *Pseudorasbora* and gibel carp *Carassius gibelio* (Záhorská and Kováč 2009, Tarkan et al. 2012) or Centrarchidae such as pumpkinseed *Lepomis gibbosus* and largemouth bass (Ribeiro and Collares-Pereira 2010).

Ecological responses, such as dietary traits, are expected to show wide plasticity in non-native species when invading new habitats (Agrawal 2001; Almeida et al. 2012; Godard et al. 2012). Accordingly, dietary traits were highly variable among the studied streams, indicating wide inter-population plasticity in bleak foraging strategies dependent on local conditions. Similarly for other biological attributes (i.e. growth and reproduction), Masó et al. (2016) suggested that this great plasticity helps to successfully invade novel Mediterranean freshwater ecosystems.

The results exposed in this chapter represent~~s~~ an interesting study on autecology of invasive bleak in the Iberian Peninsula, particularly providing insights into the population responses of this fish species to environmental conditions at the local scale. Nevertheless, the short period of this sampling (< 1 month) only reveals a snapshot of the study populations and consequently, there is the need for monitoring inter-annual variations of bleak traits. Moreover, the effect of specific environmental conditions on bleak populations is another key factor to be analysed in subsequent years. These long-term studies will provide with more accurate assessments of bleak status in Iberian freshwaters.

5. Inter-population plasticity in growth, reproduction and dietary traits of invasive bleak *Alburnus alburnus* across the Iberian Peninsula compared to a native population of France

5.1 Abstract

The bleak *A. alburnus* is an invasive cyprinid fish species in the Iberian Peninsula. Since its introduction, the bleak has displayed a strong invasive character throughout Iberian freshwaters and it is potentially dangerous for the native fish fauna, mainly via competition and aggression. However the information available on this species in the Iberian Peninsula simply addresses spatial distribution, size structure, growth rates and diet of temporary rivers of Catalonia, but there are not information about diet of this species in large rivers of the rest of the Iberian Peninsula. The aim of the present study was to assess the inter-population plasticity in dietary traits of invasive bleak across the Iberian Peninsula and a 'reference' native bleak population from France. Five Iberian rivers were surveyed (from North to South): Ebro, Tagus, Guadiana, Segura and Guadalquivir. The River Saône was sampled in France. Fish were collected from May to June 2012, just before the spawning period of bleak. All bleak individuals were measured for total length (TL, ±1 mm), from 55 to 199 mm, and dissected. Only the anterior one-third of the intestinal tract was preserved in 4% formalin for subsequent examination to avoid food remains severely digested. For dietary data we calculated percentage of occurrence and the percentage of ingested mass per food category, ingested mass (mg), prey richness (*S*), trophic diversity (Shannon index, *H'*) and trophic niche breadth (Levin index, *B*). Flying insects and detritus were the most common food categories in occurrence. In terms of ingested mass, detritus was the most important food category in Guadalquivir, Ebro and Tagus respectively. Significant differences were found between bleak populations for ingested mass, prey richness, trophic diversity and trophic niche breadth. Therefore, bleak could deeply shift the foraging strategy to better thrive in stronger flowing waters, where zooplankton is a more limited trophic resource.

5.2 Results

In total, 1200 individuals (n = 100 individuals × 2 sites × 5 Iberian rivers + 200 individuals × 1 site from the River Saône) were collected and processed. Body size ranged from 44 to 199 mm TL. In order to analyse growth and reproduction traits, significant differences were found between the six examined populations for back-calculated TL at Age 1 ($F_{5,1194}$ = 32.89; P < 0.001), Age 2 ($F_{5,896}$ = 41.41; P < 0.001) and Age 3 ($F_{5,517}$ = 27.66; P < 0.001). Only Tagus population was different for back-calculated TL at Age 4 ($F_{5,189}$ = 12.47; P < 0.001) (Table 5.1). Significant differences were found between populations for GI ($F_{5,1194}$ = 20.41; P < 0.001) (Table 5.1). Body mass ranged from 0.65 to 53.74 g eW. Bleak populations were different for BC ($F_{5,1193}$ = 7.12; Table 5.1). Regarding reproductive traits, ovary mass ranged from 0.22 to 6.69 g. Significant differences were found among populations for RI ($F_{5,328}$ = 21.51; P < 0.001) (Table 5.1). Absolute FEC ranged from 732 to 12 380 eggs. Significant differences were found among populations for FEC ($F_{5,328}$ = 16.89; P < 0.001) (Table 5.1). Oocyte size ranged from 0.73 to 1.41 mm ED. Differences were found between populations for ED ($F_{5,328}$ = 14.34; P < 0.001) (Table 5.1). The highest growth rate and best body condition, as well as the lowest gonad weight and egg size, were observed in the River Tagus. Whereas the greatest mean gonad weight and mean egg size were achieved in the River Ebro, where fecundity decreased to the lowest value found for all Iberian rivers. Also, the River Segura showed the lowest growth rate and the highest fecundity.

The youngest mean AaM was found in the River Ebro, whereas the oldest mean for this population trait was found in the River Segura (Table 5.1). The smallest mean LaM was found in the River Ebro, whereas the largest mean was found in the River Saône (Table 5.1). All the bleak populations were dominated by males, with

SR being lower in the rivers Segura and Saône, whereas the rivers Guadalquivir, Tagus and Guadiana comprised a second group with higher SR values. The River Ebro showed an intermediate SR value between these two groups (Table 5.1).

The occurrence of endemic fish prey (i.e. barbels, nases, chubs) has been continuously decreasing in the main Iberian rivers for the past decades (Maceda-Veiga et al. 2010), with bleak currently being a pivotal trophic resource in these freshwater ecosystems.

Regarding to dietary traits Diptera larvae (e.g. taxonomic Families Chironomidae and Simuliidae), zooplankton (e.g. water fleas) and detritus were the most frequent food categories for the reference native population (i.e. the River Saône), both in terms of occurrence and ingested mass (Table 5.2). The Ebro population showed a similar diet composition as per native bleak, although Ephemeroptera and Plecoptera nymphs were also important prey items. For the rest of study rivers, percentages for other categories were highly variable (Table 5.2). More in detail to be highlighted per river, vegetation and other benthic invertebrates (e.g. freshwater snails) were frequent food categories in the River Tagus. Flying insects (e.g. wasps, mosquitoes or butterflies) and Trichoptera larvae were very common in the Guadiana and Segura populations. The highest ingested mass (19%) was for nektonic insects (e.g. water boatmen or predatory beetles) in the River Guadalquivir. Detritus was an important food category for all bleak populations: 21–42% in occurrence and 13–27% as ingested mass (Table 5.2).

Regarding dietary parameters, significant differences were found between bleak populations for ingested mass ($F_{5,503} = 32.94$; $P < 0.001$). Four distinct groups were found (from low to high adjusted mean): Saône/Segura (≈12 mg), Guadalquivir (≈25 mg), Guadiana (≈40 mg) and Tagus (almost 60 mg) (Fig. 5.1A). Differences were also found for prey richness ($F_{5,503}$ = 18.68; P < 0.001). Two river groups were found: Ebro/Saône (≈3–4 prey items) and Guadiana/Guadalquivir/Tagus (≈5 prey) (Fig. 5.1B). Significant differences were found between populations for trophic diversity ($F_{5,503}$ = 19.76; P < 0.001). Two river groups were found: Saône/Ebro/Guadalquivir (H' ≈ 1) and Guadiana/Tagus (H' > 1.5) (Fig. 5.1C). Differences were found between rivers for trophic niche breadth ($F_{5,503}$ = 20.49; P < 0.001). Two distinct groups were found: Saône (B ≈ 1.4) and Tagus/Guadiana (B > 2). The remaining three rivers showed intermediate B values (1.6–1.7) (Fig. 5.1D).

Table 5.1. Population traits ofin growth and reproduction of bleak *Alburnus alburnus* in each study river (from north to south), including back-calculated total length (TL, mm) at Age i (Ai), growth index (GI, %), body condition (BC, eviscerated weight (eW), g), reproductive investment (RI, gonad weight (gW), g), fecundity (FEC, number of oocytes), egg diameter (ED, mm), age at maturity (AaM, years), length at maturity (LaM, mm TL) and sex ratio (SR, male : female)

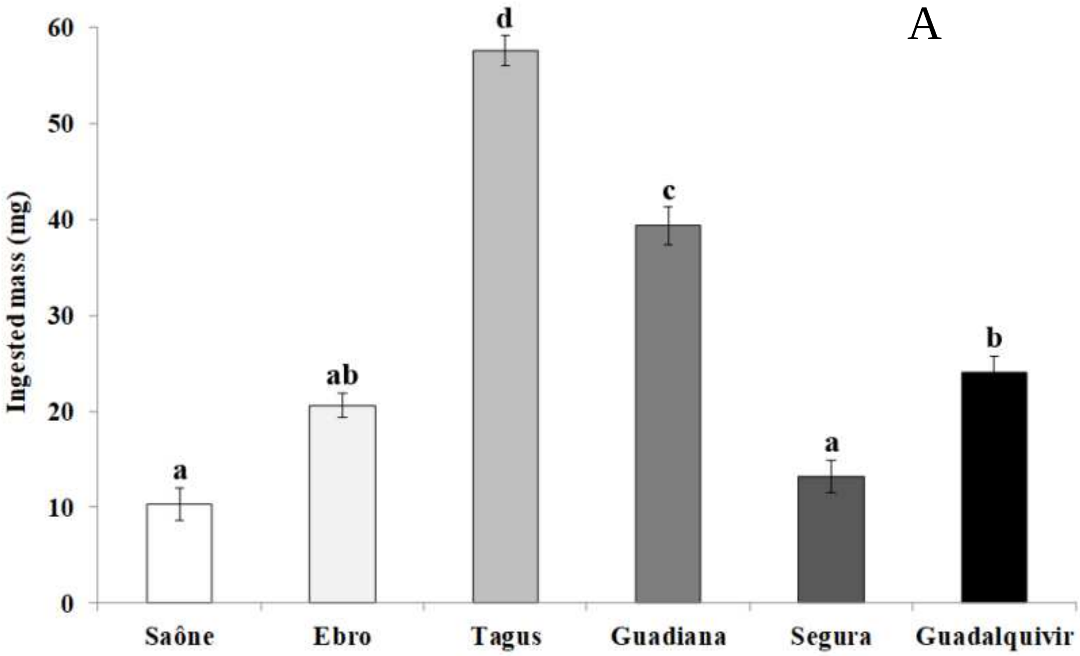
River	A_1	A_2	A_3	A_4	GI	BC	RI	FEC	ED	AaM	LaM	SR
Saône	66.8 ± 1.2^{c}	100.4 ± 1.5^{b}	111.7 ± 1.4^{ab}	113.0 ± 4.3^{a}	100.9 ± 0.8^{ab}	12.59 ± 0.70^{b}	2.48 ± 0.09^{c}	1829 ± 382^{a}	1.14 ± 0.02^{c}	2.29	95.8	1.53^{a}
Ebro	62.6 ± 1.4^{bc}	94.5 ± 2.0^{b}	110.8 ± 1.8^{ab}	113.6 ± 3.1^{a}	93.3 ± 0.7^{a}	11.19 ± 0.70^{ab}	2.54 ± 0.10^{c}	1864 ± 413^{a}	1.18 ± 0.02^{c}	1.44	80.4	2.08^{ab}
Tagus	86.5 ± 1.3^{d}	128.5 ± 2.1^{c}	148.2 ± 1.8^{c}	170.2 ± 3.6^{b}	120.1 ± 0.5^{c}	17.58 ± 0.77^{c}	1.02 ± 0.15^{a}	3162 ± 484^{b}	0.95 ± 0.04^{a}	2.18	85.0	3.17^{b}
Guadiana	69.9 ± 1.4^{c}	99.5 ± 1.5^{b}	118.6 ± 1.5^{b}	121.5 ± 3.2^{a}	106.3 ± 0.9^{b}	10.38 ± 0.68^{ab}	1.44 ± 0.12^{b}	2800 ± 496^{ab}	1.05 ± 0.02^{ab}	2.14	90.8	3.35^{b}
Segura	48.7 ± 1.8^{a}	74.8 ± 1.7^{a}	102.0 ± 1.1^{a}	116.4 ± 3.4^{a}	92.4 ± 0.6^{a}	12.80 ± 0.67^{b}	1.68 ± 0.09^{b}	8069 ± 364^{d}	1.11 ± 0.03^{bc}	2.87	92.0	1.41^{a}
Guadalquivir	56.2 ± 1.5^{ab}	91.7 ± 2.2^{b}	107.1 ± 1.2^{ab}	118.4 ± 4.9^{a}	100.0 ± 0.5^{ab}	8.31 ± 0.69^{a}	1.69 ± 0.13^{b}	5085 ± 527^{c}	1.02 ± 0.03^{ab}	2.19	83.3	3.00^{b}

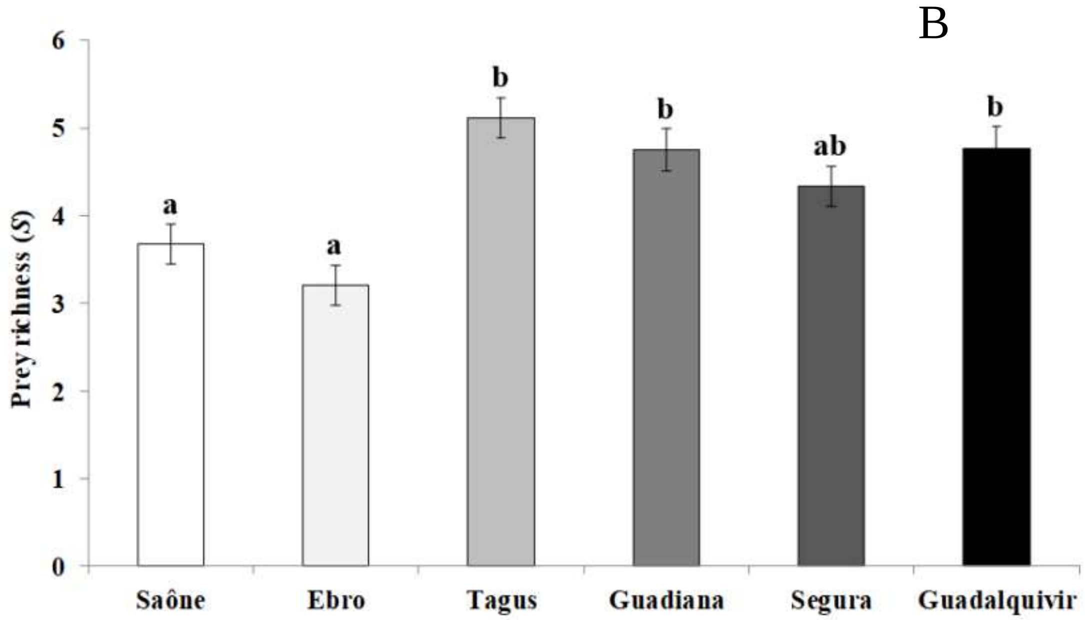
Reported values are means (A_i, GI) or adjusted means (BC, RI, FEC, ED) ± SE, after ANOVAs or ANCOVAs (see details in Materials and methods section). Significant differences among river populations are indicated by different lower-case superscript letters, after Tukey HSD and χ^2 (only for SR) tests (P < critical P-value from Bonferroni correction)

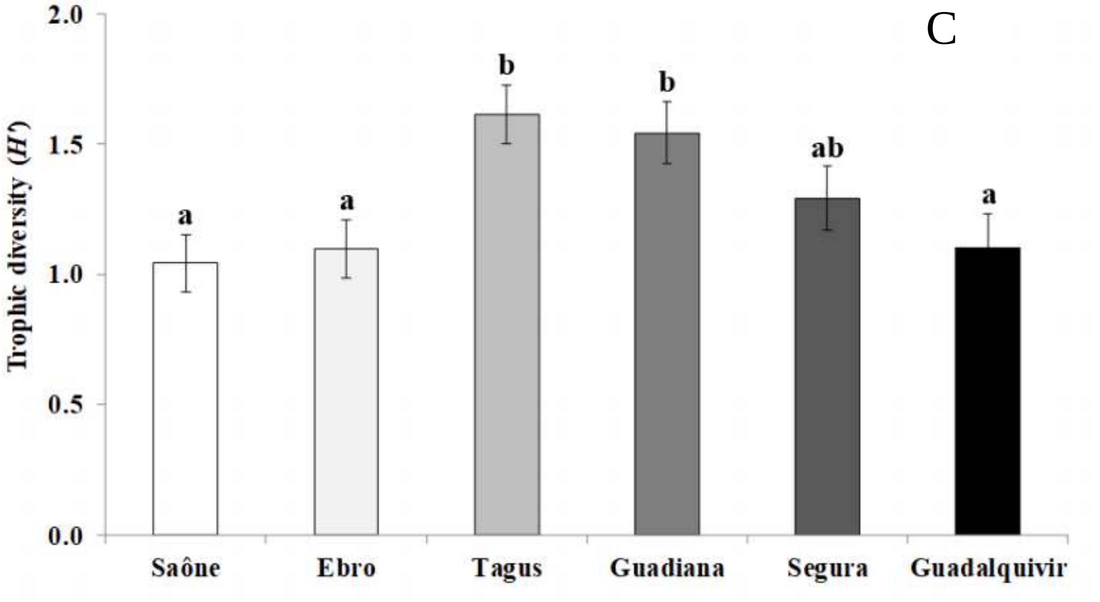
Table 5.2 Diet composition of bleak *Alburnus alburnus* in the study rivers. Percentages of occurrences (Oc., %) and ingested masses (Mass, %) are presented.

River:	**Saône**		**Ebro**		**Tagus**		**Guadiana**		**Segura**		**Guadalquivir**	
Food category	**Oc.**	**Mass**	**Oc.**	**Mass**	**Oc.**	**Mass**	**Oc.**	**Mass**	**Oc.**	**Mass**	**Oc.**	**Mass**
Algae and plant debris	3	<1	2	5	29	17	5	3	3	<1	8	9
Zooplankton[a]	50	17	5	15	11	8	1	<1	5	3	10	6
Ephemeroptera and Plecoptera nymphs	5	9	33	21	5	10	20	23	7	21	5	12
Odonata nymphs	1	2	11	6	1	5	5	9	1	<1	1	3
Diptera larvae	69	39	27	15	3	15	3	1	59	31	3	16
Trichoptera larvae	6	1	5	<1	1	<1	15	13	6	11	1	5
Other benthic invertebrates[b]	12	4	3	3	53	13	1	<1	12	1	71	14
Nektonic insects[c]	1	<1	1	<1	1	<1	1	<1	1	<1	20	19
Flying insects[d]	3	<1	5	8	35	7	61	23	3	19	-	-
Terrestrial arthropods[e]	1	<1	1	<1	-	-	1	9	1	<1	7	2
Detritus	31	27	25	26	42	24	24	18	31	13	21	14

[a] Acari, Cladocera and Copepoda; [b] Mollusca, Oligochaeta and Coleoptera larvae; [c] Corixidae and Coleoptera adults; [d] Hymenoptera, Diptera and Lepidotera; [e] Araneae and Formicidae.


A
Ingested mass (mg)
0
10
20
30
40
50
60
a
ab
d
c
a
b
Saône
Ebro
Tagus
Guadiana
Segura
Guadalquivir


B
Prey richness (S)
0
1
2
3
4
5
6
a
a
b
b
ab
b
Saône
Ebro
Tagus
Guadiana
Segura
Guadalquivir



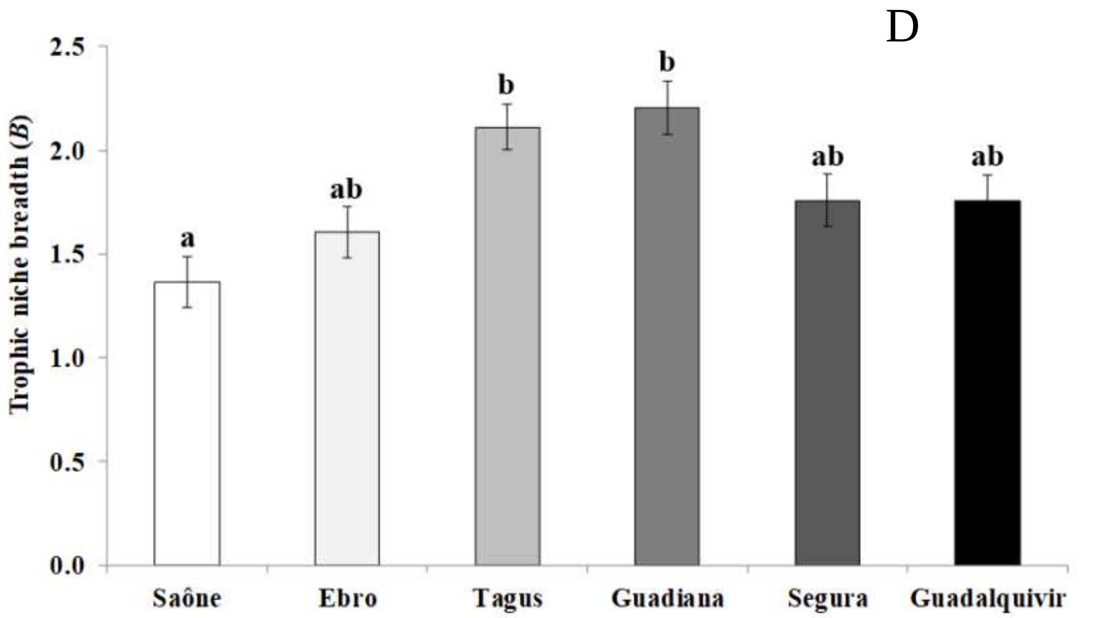


Figure 5.1. Comparison of four dietary parameters for bleak among studied rivers (A, B, C and D). Results are adjusted means ± SE, after ANCOVAancova (covariate: TL). Sample size (*n*) = 326. Sampling period: May–June 2012. Letters above columns indicate significant differences in bleak populations, after Tukey HSD tests ($P \leq 0.05$)

5.3 Discussion

The population traits of invading species can change when adapting to novel environments (Sakai et al., 2001), displaying high phenotypic variability, which facilitates establishment success (Agrawal, 2001). Such ‘flexibility’ has been clearly observed in several non-native freshwater fishes introduced to the Iberian Peninsula (Ribeiro et al. 2008). In the present study, bleak populations demonstrated considerable shifts in growth and reproductive traits across nearby river catchments, which corroborates the results of previous, smaller-scale studies of bleak in the Iberian Peninsula (Almeida et al. 2014; Masó et al. 2016), but also reflects the species’ phenotypic plasticity more widely across its native range. For example, bleak populations in the rivers Danube in Serbia (Lujić et al. 2013) and Pilica in Poland (Mann and Penczak 1984) were found to have greater back-calculated TLs at age 4 (140 and 160 mm respectively the latter converted from SL) than observed in the River Saône (Table 5.1), indicating faster growth rates. Whereas, body condition ‘*K*’ (i.e. plumpness) in the river Danube and Pilica were lower, ≈20 and ≈10% respectively, than in the River Saône (Table 5.1). In the Danube, Lujić et al. (2013) also observed a higher sex ratio (2.27) than that found in the River Saône (Table 5.1). Bleak reproductive investment (GSI) in the River Meuse, Belgium (Rinchard and Kestemont, 1996), was reported to reach almost 18%, a slightly higher value than that (≈15%) in the River Saône. Bleak fecundity estimates also vary across the species’ native range, with ≈6400 eggs reported for 5-year-old females from the River Thames, UK (Mackay and Mann 1969), which is almost double the mean of ≈3800 eggs was estimated in 4-year-old females from France. Egg size also varies in bleak, with a mean diameter of 1.48 mm for various water courses in Poland (Bonisławska et al. 2001), which is much higher than observed in River Saône bleak

(≈1.20 mm; adjusted to 1.14 in the present results). Overall, these data on reproductive traits suggest that bleak populations in France displayed a lower reproductive capacity (ovary mass, fecundity and egg size) than in the invasive Iberian population, which may be due to differences in latitude, with this being used as a surrogate for temperature regime (e.g. Cucherousset et al. 2009). Indeed, TL at maturity increases significantly (LaM = 1.449Lat. + 30.664, $r^2 = 0.606$, $P = 0.04$) with increasing latitude (data from Table 5.1 combined with data for the British Isles, (Froese and Binohlan 2003) whereby the mid-point (110 mm TL) of the reported range 100–120 mm TL and the latitude for the River Thames at London were used; no such relationship was found with AaM and latitude, which contrasts the significant relationship between AaM and latitude observed in European populations of non-native pumpkinseed *Lepomis gibbosus* (Copp and Fox 2007).

Bleak from the rivers Saône and Ebro (the closest Iberian river to the native range) had similar population responses (i.e. growth and reproductive traits), except for age and length at maturity. The River Saône is located in an area under the most common climatic conditions for the bleak native range in Europe (Keith et al. 2011). The warmer Mediterranean climate of the River Ebro may promote earlier sexual maturity in bleak. This resembles the elevated juvenile growth of crucian carp, *Carassius carassius*, under the ‘benign’ weather conditions of England relative to other continental European locations (Tarkan et al. 2016). Bleak sex ratios shifted to males in all the study rivers, which may have been the result of a greater predatory pressure on females (i.e. more ‘profitable’ prey) owing to their ‘nutritious content’ (eggs and ovaries) and particular behavioural traits, e.g. higher ambulation rate (Almeida et al. 2014). With the exception of the River Segura (more similar to the native population), the effect of this predation may be particularly exacerbated in

Iberian freshwaters, where a high abundance of both non-native and native piscivorous species co-exist (e.g. invasive fishes, cormorants, herons and otters). Indeed, linear latitudinal gradients have been found in certain growth and reproductive traits of native and introduced freshwater fishes throughout Europe, such as pumpkinseed (Copp and Fox 2007; Cucherousset et al. 2009), black bullhead *Ameiurus melas* (Copp et al. 2016) and crucian carp (Tarkan et al. 2016). In these studies, latitudinal variation was explained as a surrogate of temperature regime. However, no clear pattern of increase or decrease was observed in bleak populations, except in mean TL at maturity, and this relationship was only valid if the estimated mean TL at maturity for the British Isles was included in the regression analysis. This otherwise lack of a relationship was probably due to the limited latitudinal amplitude, i.e. 37–47ºN, against 47–59ºN in Cucherousset et al. (2009) and 50–62ºN in Tarkan et al. (2016) based on a greater number of study sites (*n* = 10 and 2, respectively). Consequently, it would be even more difficult to find any spatial pattern in the examined population traits. Regarding the time since introduction, a pan-European study of pumpkinseed found that more recently established populations (<50 years since establishment) appear to exert greater reproductive effort than those that have been established longer (Copp and Fox 2007). According to the available information on bleak (e.g. Vinyoles et al. 2007), this species was sequentially introduced into the study Iberian rivers and, thus, their populations will potentially reflect particular ‘establishment stage’ on the examined traits. Similarly, as per the latitudinal variation, the period since introduction was much shorter in the current study (<15 years between the rivers Ebro and Guadalquivir) and this temporal range may not be a sufficiently-long time-frame within which to reveal a pattern. Another potential explanation is that the establishment stage had been reached in a few years after bleak introductions (e.g. Bøhn et al. 2004) and given that the surveys were conducted in 2012 (more than 6 years from first mention for the last invaded river), the ‘time since introduction’ effect may be ‘diluted’. In relation to the dietary traits, given that bleak mainly inhabits still-waters in its native area, this species is well-adapted to play an ecological role as an open-water feeder, with diet being chiefly based on zooplankton (Vinni et al. 2000; Vašek and Kubečka 2004). Thus, planktonic Crustacea were very important for bleak in the River Saône, although benthic Diptera larvae and detritus were more consumed. This difference between scientific literature and the present results was probably because of most surveys for bleak have been carried out in lakes and reservoirs (e.g. Vinni et al. 2000; Vašek and Kubečka 2004), whereas bleak diet has received less attention in flowing-waters. But even in lentic environments, bleak can modulate this zooplanktivorous strategy at each particular habitat patch to increase resource partitioning and consequently, reduce competition with co-existing fish species, such as roach *Rutilus rutilus* or freshwater bream *Abramis brama* (Vinni et al. 2000; Vašek and Kubečka 2004). Dietary indices were more similar between the River Saône and the River Ebro, the closest (geographically) study area to the reference population. This was also observed by Latorre et al. (2018) for other population responses (i.e. growth and reproduction), which was explained in terms of climatic ‘proximity’. In the main Iberian rivers, bleak showed a high consumption of detritus, which was also found elsewhere within this eco-region, from streams to reservoirs (Latorre et al. 2016; Almeida et al. 2017). More specifically, other food items were prominent among the study populations, including a variety of contrasting ecological features for each trophic resource: vegetation, neuston, nekton and benthos. Thus, consumption of algae and plant material was higher in the River Tagus. In this respect, Vinni et al.

(2000) demonstrated that intra- and mainly inter-specific competition provided a potential explanation to understand this result (i.e. high use of vegetation) in the native area. However, Latorre et al. (2018) did not find any effect of this ‘low nutritious’ feeding type (detritus and vegetation) or competition on the growth rate in the River Tagus, with this population showing the highest values. In the Guadiana and Segura populations, bleak took advantage of prey from the water surface, such as fallen arthropods, which results in a low effort in terms of energy investment. Bleak also caught prey from the water column in the River Guadalquivir, but this population displayed a ‘costlier’ foraging alternative, as nektonic insects (e.g. taxonomic Family Dytiscidae) require higher pursuit and capture efforts. But more importantly, bleak fed on a great variety of benthic prey, from nymphs and larvae to molluscs and annelids, in all river populations. This finding indicates an elevated capacity of this fish to use food resources apparently less suitable to its morphological adaptations (e.g. conspicuous superior mouth). In overall, these results suggest that bleak could deeply change the foraging strategy, from a ‘pelagic’ to a ‘benthic’ feeder, which may aid bleak to better thrive at each Iberian river, where zooplankton availability may be a more limited trophic resource (Almeida et al. 2017). Such a significant dietary shift has been also observed in the Iberian Peninsula for other invasive fishes adapted to a more stable hydrological regime in their native ranges, although they are phylogenetically and geographically very ‘distant’ (e.g. taxonomic Family Centrarchidae from North America, see Almeida et al. 2012).

Regarding dietary parameters, variation limits for the four examined predictors were similar as per other study areas in the Iberian Peninsula (Latorre et al. 2016 in the NE; Almeida et al. 2017 in the SW). The present data were obtained from large rivers and the just mentioned studies included streams and reservoirs. Thus, these overall results indicate that all Iberian bleak populations possess the same capacity of adapting to the particular habitat conditions where this invasive fish inhabits. No clear pattern was found for any dietary parameter in relation to the year of introduction, which was in accordance with Latorre et al. (2018) for growth and reproduction traits. This lack of relationship is probably because the establishment stage has been reached in a few years after bleak introductions (e.g. Bøhn et al. 2004). However, foraging strategy appeared to follow a unimodal response in relation to the latitudinal gradient (Oksanen and Minchin 2002), with Guadiana and mainly Tagus showing the maximum values. A potential explanation is that bleak may be more favoured by moderate Mediterranean climate in central Iberia than Temperate conditions in the native area, whereas bleak are affected by slightly more ‘severe’ ecological conditions in southern Iberia: stronger summer drought with the consequent decrease in discharge or lower oxygen level because of high temperature (Gasith and Resh 1999). Specifically for ingested mass, Tagus River showed the highest value of this dietary parameter, resulting in only one ‘statistical group’. This could compensate a higher proportion of vegetation and detritus in the diet (see above). Indeed, Tagus population showed the highest back-calculated lengths at all ages and growth index among the same study rivers (Latorre et al. 2018). Additionally, more ‘benign’ conditions in central Iberia may improve the physiological status of bleak, which facilitates this fish species to use alternative prey and widen its diet, with the corresponding reflection in prey richness and chiefly, trophic diversity and breadth. In contrast to this ‘generalist’ strategy, more northerly and southerly populations (i.e. distant from the optimum, see Oksanen and Minchin 2002) displayed a ‘selective’ or ’specialist’ strategy, reducing the complexity of diet composition.

Ecological responses, such as dietary traits, usually show wide variability in non-native species when invading new habitats, with this being particularly clear in freshwater fishes (Copp et al. 2005; Almeida et al. 2012; Latorre et al. 2016). In the Iberian Peninsula, invasive fishes usually display wide trophic variability under contrasting environmental conditions, which contributes to their invasion process (Leunda 2010; Latorre et al. 2016; Almeida et al. 2017). Accordingly, dietary traits were clearly variable among the study rivers, indicating wide plasticity in bleak foraging strategies dependent on particular river conditions (i.e. habitat heterogeneity, food supply). Similarly as per other biological attributes (e.g. growth and reproduction), this high inter-population variability is considered as a mechanism for bleak to successfully invade novel Mediterranean freshwater ecosystems, from streams to large rivers (Masó et al. 2016; Latorre et al. 2018). Indeed, Iberian endemic species are specialized to narrow trophic niches (Reyjol et al. 2007) and this low level of co-evolutionary competition is the reason why Iberian fish fauna is not adapted to strong invasive competitors, such as bleak. This is one of the main reasons to consider this fish species as a bio-invader of high risk in the Iberian Peninsula (Almeida et al. 2013). The present work represents one of the few studies on the invasion biology of a non-native fish species, native to other parts of Europe, at a large scale in the Mediterranean region of Europe. Other large-scale studies of introduced fishes in the Mediterranean region have focused on species non-native to Europe and contiguous parts of Asia (e.g. Fox et al. 2007; Tarkan et al. 2012). Overall, the present study highlights the wide inter-population variability in growth and reproductive traits displayed by invasive bleak across the Iberian Peninsula. This variety of population strategies is probably displayed to compensate the particular environmental pressures of novel habitats and facilitate bleak establishment (e.g. see a comprehensive review in Sakai et al. 2001).Topics for future research include habitat availability, fish community composition, predatory pressure, habitat structure and water quality, in order to identify the species mechanisms for invading novel ecosystems successfully. These data may also aid to better predict and mitigate impacts exerted by this bio-invasion on the highly valuable fish fauna of Mediterranean Europe (Reyjol et al. 2007). Consequently, environmental managers should apply urgent conservation measures to control bleak populations across Iberian freshwaters. Firstly, these measures should focus on the target species (i.e. fish culling), but also include actions on aquatic habitats to restore the ‘Mediterranean conditions’ that benefit native fish communities and hamper invasive species (e.g. recovery of natural flow regimes), specifically the bleak (Matono et al. 2018).

6. Inter-population variability in physiology and morphology between wild and captive breeding populations of the endangered species *Aphanius iberus*

6.1 Abstract

Phenotypic plasticity occurs in many different phenotypes including behaviours, life-history traits, morphology and physiology, in response to abiotic and biotic factors. Captive breeding can cause changes in the morphology and physiology of fish. In this sense, we investigated differences in the morphology and physiology of wild and captive populations of an endemic fish species from the Iberian Peninsula: *Aphanius iberus* (Iberian toothcarp). This small fish is included in the IUCN list as an endangered species. Its natural range has been greatly reduced in recent decades, mainly due to the destruction of its natural habitat and the introduction of invasive species such as the *Gambusia holbrooki*. The aim of this chapter was to assess differences in swimming capacity, morphology and physiology between captive and wild populations of Iberian toothcarp. Our results showed differences in morphology and physiology between populations but not in critical swimming speed (*Ucrit*). The wild population showed higher values of SMR, MMR and AAS than the captive population. These differences could be related to environmental factors such as the presence of predators, intra-interspecific competition, and the availability of food and habitat complexity. There were also differences in the morphology of the two populations studied and between sexes. Conversely to other studies, wild population showed a more fusiform bodies than captive population which could be related to environmental factors such as habitat complexity, search for food and the presence of predators. Also the higher MMR in wild males and females was negatively correlated with some morphological traits such as narrow and elongated bodies indicating that a more fusiform body favours a greater MMR. In addition, other morphological traits such as a longer and narrower caudal fin were correlated positively with SMR for wild males and females. All these results seem to indicate that differences in the environmental conditions between wild and captive

habitats could explain morphological and metabolic changes in these populations as a result of different environmental pressures through phenotypic plasticity.

6.2 Results

6.2.1 Critical swimming speed and metabolism

In total, fifty-seven (*n* = 57) captivity-reared individuals and thirty (*n* = 30) wild individuals successfully completed swimming performance tests. Mean critical swimming speed and metabolic traits for the wild and captive populations of Iberian toothcarp were summarised in Table 6.1.

Table 6.1. Critical swimming speed and metabolic traits for the wild and captive populations of Iberian toothcarp *A. iberus*. Means and standard deviations (SD) are shown. Abbreviations are SL (standard body length), *M* (body mass, *Ucrit* (critical swimming speed), SMR (standard metabolic rate), MMR (maximuml metabolic rate), AAS (absolute aerobic scope).

	Wild				***Captive***			
	Males (*N* = 15)		Females (*N* = 15)		Males (*N* = 23)		Females (N = 34)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
S_L (mm)	2.09	0.376	2.34	0.543	2.30	0.256	2.45	0.427
M (g)	0.29	0.195	0.40	0.360	0.29	0.089	0.38	0.210
U_{crit} (cm·s^{-1})	8.17	3.477	9.05	3.316	10.66	3.866	9.92	3.780
SMR (mg O_2·h^{-1})	0.17	0.153	0.24	0.244	0.12	0.065	0.07	0.062
MMR (mg O_2·h^{-1})	0.36	0.210	0.49	0.270	0.27	0.140	0.21	0.113
AAS (mg O_2·h^{-1})	0.21	0.120	0.24	0.120	0.14	0.116	0.13	0.100

ANCOVA results (Table 6.2) showed that the interaction between SL or M and the categorical factors (i.e. population and sex) were not significant (P > 0.05), and hence homogeneous slopes among groups were assumed for the response variables. The critical swimming speed (U_{crit}) was positively correlated with SL and M and was not significantly different between populations and sexes (Table 6.2). Metabolic traits (SMR and MMR) were positively correlated with SL and M, and MMR was significantly different between populations (Table 6.2), being higher for wild populations (Table 6.1). In contrast, the interaction between population and sex was significant for SMR after accounting for SL or M (Table 6.2), thereby showing a different trend between populations and sexes. Captive males showed higher values of SMR than captive females ($F_{1,\ 53}$ = 10.81; P = 0.002) whereas sexes of wild population did not differ significantly from each other ($F_{1,\ 26}$ = 2.06; P = 0.163) when comparing individuals of similar size. Absolute aerobic scope (AAS) was not significantly correlated with SL nor M, and then an ANOVA was applied. We found significant differences between populations ($F_{1,79}$ = 12.65; P = 0.001) but not between sexes ($F_{1,79}$ = 0.02; P = 0.887). The interaction between population and sex was also not significant ($F_{1,79}$ = 1.22; P = 0.273)

.

Table 6.2. Analyses of covariance (ANCOVA) of critical swimming speed and metabolic traits for wild and captive populations of Iberian toothcarp *A. iberus*.

	U_{crit}			**SMR**			**MMR**		
	SS	d.f.	*P*	SS	d.f.	*P*	SS	d.f.	*P*
	$R^2_{\mathrm{adj}} = 0.132$			$R^2_{\mathrm{adj}} = 0.266$			$R^2_{\mathrm{adj}} = 0.356$		
M	0.340	1	***	1.886	1	***	0.567	1	**
Population	0.028	1	0.287	1.537	1	***	3.162	1	***
Sex	0.150	1	0.436	0.028	1	0.656	0.024	1	0.584
Population × Sex	0.014	1	0.449	1.274	1	**	0.267	1	0.069
Residuals	2.018	83		11.321	81		6.321	80	
	SS	d.f.	*P*	SS	d.f.	*P*	SS	d.f.	*P*
	$R^2_{\mathrm{adj}} = 0.143$			$R^2_{\mathrm{adj}} = 0.258$			$R^2_{\mathrm{adj}} = 0.354$		
SL	0.365	1	***	1.771	1	***	0.547	1	**
Population	0.009	1	0.551	1.777	1	***	3.294	1	***
Sex	0.230	1	0.335	0.033	1	0.631	0.027	1	0.564
Population × Sex	0.010	1	0.510	1.284	1	**	0.270	1	0.069
Residuals	6.302	77		11.436	81		6.342	80	

Models test differences between populations and sexes with fish mass (M) and standard length (SL) as covariates. All variables were $\log_{10}$-transformed (* $P \le 0.05$, ** $P \le 0.01$, *** $P \le 0.001$). See Table 6.1 for abbreviations of response variables.

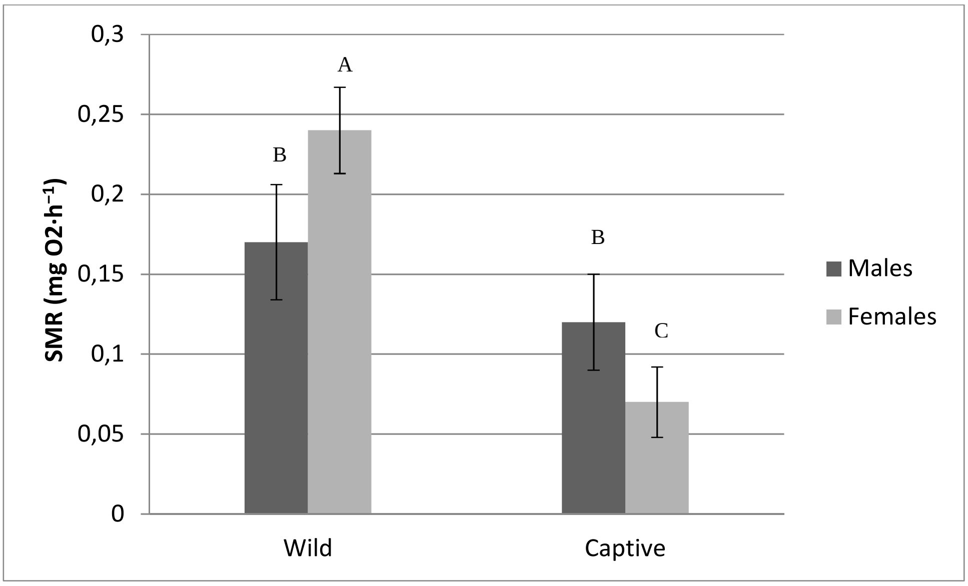


Figure 6.1. Adjusted means and standard errors (SE) of the Standard Metabolic Rate (SMR) for populations and sex of the Iberian toothcarp. Capital letters A, B and C respresent significant mean differences between groups.

6.2.2 **Body shape analysis**

MANOVA performed on the matrix of 18 partial wraps showed morphological differences between populations and sex but no interaction between population and sex was found (Table 6.3).

Table 6.3. Summary of MANOVA of the effects of population (C = captive population; W = wild population), sex (M = Male; F = Female) and their interaction on body shape variation of *A. iberus*. Variation was measured in 18 partial warps for each individual. For each test, df = 14 (effect) and 54 (error).

Source of variation	**Wilk's λ**	**F**	**P-value**
Population (C or W)	0.475	4.191	< 0.001
Sex (M or F)	0.476	4.170	< 0.001
Population × Sex	0.651	2.029	0.069

DFA analysis showed significant differences between groups (population and sex combinations) in axis one (Wilk's $\lambda = 0.145$; $\chi^2 = 117.801$; $P < 0.001$) and axis two (Wilk's $\lambda = 0.382$; $\chi^2 = 58.742$; $P < 0.001$). The first axis of DFA explained 57.7% of the variation in morphology between groups and the second one explained 32.9% (Figure 6. 2). Cross-validation matrices generated from the DFA indicated that 65.5% of captive females ($n = 19$), 73.7% of captive males ($n = 14$), 50% of wild females ($n = 7$) and 66.7% of wild males ($n = 6$), were correctly classified *a priori*.

The first DFA axis (DF1) primarily revealed differences in the x coordinate of the posterior anal fin insertion (DF loading = –0.468), the x coordinate of the beginning of the operculum (DF loading = 0.338), the y coordinate of the posterior dorsal fin insertion (DF loading = 0.302), and the y coordinate of the dorsal head end (DF loading = 0.157). The second DFA axis (DF2) was mainly associated with differences in the x coordinate of the anterior anal fin insertion (DF loading = –0.572), the x coordinate of the dorsal head end (DF loading = 0.462), the x coordinate of the ventral caudal fin insertion (DF loading = 0.453), the x coordinate of the dorsal caudal fin insertion (DF loading = 0.369), the x coordinate of the posterior dorsal fin insertion (DF loading = –0.205) and the y coordinate of the beginning of the operculum (DF loading = 0.171). To interpret the first two DFA axis we only considered landmark coordinates with significant loadings ($P < 0.05$).

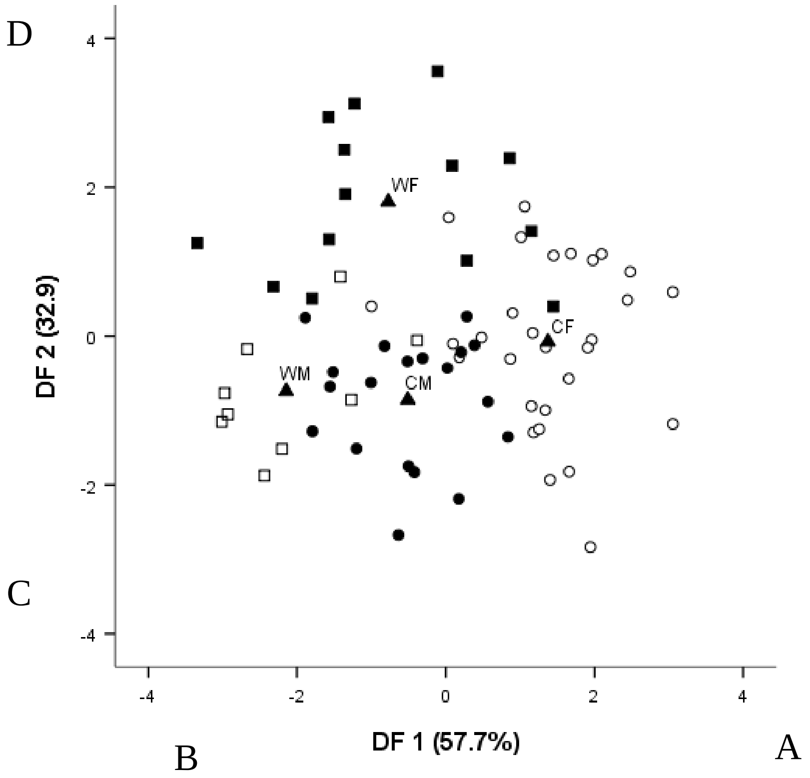


Figure 6.2. Ordination of all *A. iberus* specimens along the first two discriminant function axes from discriminant function analysis on partial warps data. Legend: white circle (CF, captive females), black circle (CM, captive males), black square (WF, wild females), white square (WM, wild males). Black triangles represent centroids. Letters A, B, C and D represent positive and negative extremes of the observed shape variation associated with DF1 and DF2 axes; N = 87 (see figure 6.3).

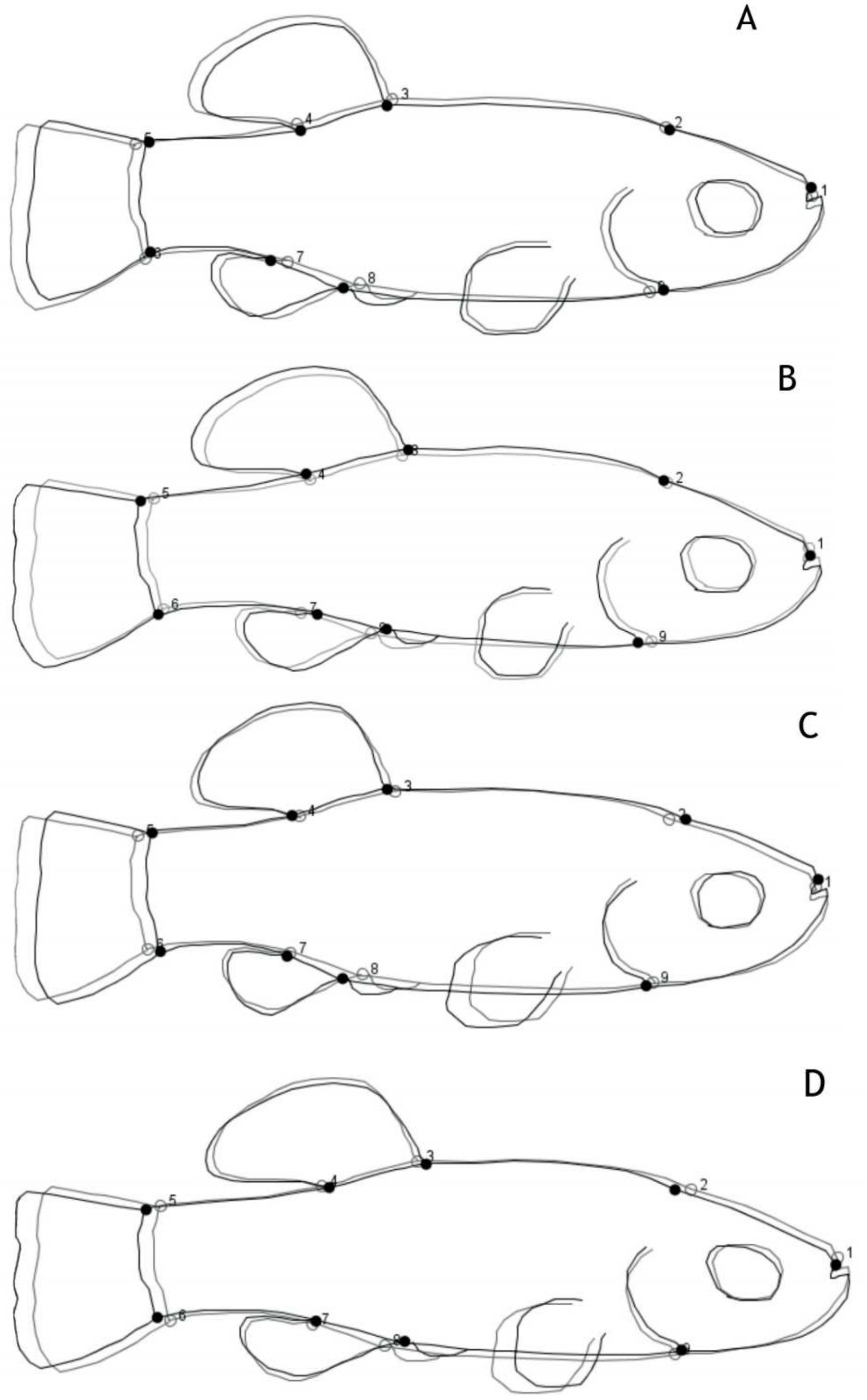


Figure 6.3. Shape comparisons of Iberian toothcarp populations between consensus shape and each extreme value of the DFA axes. Consensus shape (gray line), mean of each extreme DFA value (black line).

Captive females were characterized by shorter heads, shorter and wider caudal peduncles and deeper bodies. By contrast, wild males showed longer heads, longer and narrower caudal peduncles and narrower bodies. Wild females and captive males displayed an intermediate morphology along this axis. On the other hand, wild females were characterized by shorter heads, shorter caudal peduncles and narrower bodies. These results were supported by the deformation grid plots (Figure 6.3), which reflect the degree and type of shape change between the consensus form and the form of each group (population by sex combinations) analysed.

6.2.3 **Critical swimming speed, metabolism and body shape**

The correlation of individual DFA scores with *Ucrit* and AAS revealed that there was not significant correlation between morphology and these traits, whereas MMR and SMR showed significant correlation with morphology (Table 6.4). Specifically, MMR was mainly correlated with DF1 whereas SMR was mainly correlated with DF2 (Table 6.4). Therefore, it seems that morphological attributes conferring a more shortened and gibbous body shape may allow captive females to display lower MMR than wild males, whereas a more fusiform body shape may allow wild females to display higher SMR than the other groups compared.

Table 6.4. Pearson's linear correlations between *Ucrit*, MMR, SMR and AAS with DF1 and DF2 of the discriminant function analysis (DFA). Variables were logarithmically transformed (log10). (p values in parenthesis).

	Ucrit	**MMR**	**SMR**	**AAS**
DF1	0.026 (0.833)	-0.438 (0.001)	-0.231 (0.054)	-0.181 (0.136)
DF2	-0.186 (0.120)	0.320 (0.050)	0.341 (0.010)	0.149 (0.223)

6.3 Discussion

In this study, we compared swimming capacity and metabolic and morphological traits between captive and wild populations of *A. iberus*. We found differences between populations in metabolic traits such as SMR, MMR and AAS; and in morphology between populations and sexes but not for *Ucrit* between populations and sexes. Several studies have shown that phenotypic plasticity occur in many different phenotypes including behaviour, physiology, life-history traits, and morphology, and in response to different types of abiotic and biotic factors (Oufiero and Withlou, 2016). There is evidence that metabolic rates can change in response to environmental factors such as food availability (Burton et al. 2011; Auer et al. 2015; Metcalfe et al. 2016), diet quality (Naya et al., 2007; Killen et al. 2012; Norin et al, 2015), temperature (Killen et al. 2010; water salinity (Allan et al. 2006; Dalziel et al. 2012; Norin et al. 2016) and presence of predators (Fu et al. 2015; Auer et al. 2018). Several studies have documented that SMR can decrease when fish are subject to a period of food restriction (Du Preez, 1987, Wieser et al., 1992, Auer et al., 2015) and can increase when food supplied above reference levels (O'Connor et al., 2000; Van Leeuwen et al., 2011, 2012; Auer et al., 2015). Since the captive rearing population was maintained in outdoor tanks (semi-controlled mesocosms) and was not artificially fed during their captivity, food availability and diversity was expected to be more limited than that of the wild population. Consequently, SMR differences between populations could be related to differences in their recent energy intake history (Rosenfeld et al., 2015). Further, it has been shown before that the presence of predators in a given habitat can induce prey fish to show higher SMR than those that live in lacking predators environments (Fu et al., 2015; Auer et al., 2018). For example, Fu et al. (2014) showed that the presence of predators increased SMR in individuals of pale carp *Zacco platypus*, which can improve its rapid response to avoid being preyed. Then, the observed differences in SMR in our study could be also attributed to differences in predation pressure among wild and captive habitats.

Although intraspecific variation in MMR and AAS have not been as studied as SMR they seem to show similar levels of intraspecific variation as SMR (Metcalfe et al., 2015). Our results of MMR showed that the wild population, that inhabited a high salinity lagoon, had the highest values, whereas captive population reared mainly in freshwater showed lower values. Similarly, Dalziel et al. (2012) have found differences in MMR and AAS when comparing three-spined sticklebacks *Gasterosteus aculeatus* from populations experiencing different osmoregulatory demands. In addition, Prakoso et al. (2018) observed that in rock bream *Oplegnathus fasciatus*, salinity concentration showed a positive correlation with MMR. Therefore, our results suggest that differences in water salinity between habitats could be one of the causes that would explain the differences observed in MMR and AAS between wild and captive populations. In the other hand, as Metcalfe et al. (2016) pointed out, MMR variation could also be related to the ability to catch prey and avoid predators. In the same way, the highest MMR showed by wild population in our study suggested that this might be related to both avoid predation and food search. In addition, (Farrell 2016) suggested that AAS is important for events such as predator-prey interactions or to overcome hydrologic alterations (i.e. floods). As explained above, in outdoor tanks where the fish were bred in captivity there was no predators nor hydrologic alterations, and then the highest values of AAS found in the wild population could be related to the presence of predators and natural floods (Alcaraz et al. 2008).

About the effects of food availability on MMR and AAS, in our study we found that captive population which was subject to low food availability showed lower MMR and AAS than wild population. In contrast, Van Leeuwen et al. (2011) did not find any relationship between MMR and food levels in *Onchorhynchus mykiis* or in *Onchorhynchus kisutch*, although they found a negative relationship between AAS and food availability. In addition, Killen et al. (2014) found a relationship between high SMR and reduced AAS and no effect on MMR when comparing two populations of *Phoxinus phoxinus* under different conditions of food availability. Furthermore, Auer et al. (2016) showed a correlation between availability of food and SMR and AAS in brown trout *Salmo trutta*, but not with MMR. Conversely, Biro and Stamps (2010) observed a positive correlation between SMR, MMR and higher resource intake rates. Thus, differences in MMR and AAS between populations in our study may be related, at least in part, to differences in habitat conditions such as presence of predators, water salinity concentration and food availability, although we do not rule out that other environmental factors may also influenced the MMR and AAS.

Some studies that checked SMR differences between males and females, showed that males had higher SMR than females, being these differences attributed to growth differences due to food intake and to activity differences between males and female (Lucas et al. 1993; Madenjian et al. 2016). In contrast, Srean et al. (2017) found no sex-dependent differences in SMR for mosquitofish but they found that MMR was significantly higher for males than females. They argued that females may have a higher metabolic expenditure due to physiological reproductive investment but males may be investing more energy in locomotion while searching for breeding partners or displaying territorial behaviours (Kolok 1999) thus compensating for potential sexual differences in resting conditions.

However, our study showed that after accounting for the effect of mass, wild females had higher SMR than wild males and captive males had higher SMR than captive females. Some studies have been argued that environmental fluctuations due to seasonality (Madenjian et al. 2016) could play an important role in explaining sexual differences in SMR, but unfortunately, in our study we did not account for these differences, so further studies about it would be necessary.

Iberian toothcarp is sexually dimorphic being males smaller than females (Oliva-Paterna et al., 2006). Although it is expected that species that show sexual dimorphism could also show differences in swimming speeds (Nicoletto 1991; Royle et al 2006, Oufiero and Garland 2007), we did not find differences in *Ucrit* between populations or sexes.

In this study we also found morphological differences between populations and sexes. It is known that intraspecific morphological variation in a variety of fish species is related to habitat differences, diet, and other factors (Moles et al., 2010). Morphological differences are expected to occur when habitats require specific abilities, such as fast vs. slow swimming, or slow and precise manoeuvring vs. cruising; and the magnitude of these differences should be related to the degree of differences among habitats (Bourke et al., 1997). Although Iberian toothcarp is a species that can live in a wide variety of brackish and freshwater habitats (Garcia-Berthou and Moreno-Amich, 1999), it is considered a species that prefer complex habitats with abundant submerged vegetation and slow current flow (Vargas Pera, 1993; Alcaraz et al. 2008; Magellan and García-Berthou 2016), and species that live in these types of habitats usually show morphological traits related to unsteady swimming such as deep bodies through the caudal region, medium fins and large caudal fins with low aspect ratios (broad surface area powerful thrust; but high frictional drag) (Blake, 1983; Walker, 1997; Domenici et al., 2008). Unsteady swimming is useful when fish have to make frequent fast starts, rapid turns, and quick stops. These complex manoeuvres are useful for escaping predators, capturing prey, and navigating through complex habitats (Langerhans, 2009). Our results showed that wild population had a more fusiform morphology, narrower heads, narrower bodies, forward placement of the anal fins, and narrower and longer caudal peduncles than captive population. As we said before, wild habitat was more complex than the captive one and it is well known that habitat complexity can induce morphological changes in fishes. For example, Garduño-Paz et al. (2010), when compared two three-spined stickleback *Gasterosteus aculeatus* populations exposed to different habitat complexity, they found that complex habitat induced more streamlined shape and smaller heads, which they related to feeding behaviour. Similarly, Black et al. (2017), who compared captive and wild populations of *Cyprinodon bovinus*, suggested that a simplified environment could have altered the diversity and location of the prey in the captive environment and it would cause a modification of the feeding behaviour and, consequently, a change in the position of the head. All these results suggest that morphological differences between populations could be related to differences in habitat complexity.Differences in predation pressure could also partly explain morphological differences among wild and captive populations because it is well known that the presence of predators induce morphological changes in fishes (Chivers ert al., 2007; Eklöv and Jonson, 2007). For instance, Ingley et al. (2014) compared morphologies between three species of the genus *Brachyrhaphis* from different habitat types (e.g. with and without predators) and they concluded that the presence of predators can modify the shape of fish of the same species. Although a shortened and deeper caudal peduncle may indicate a greater capacity to accelerate at the expenses of longer steady swimming and to enhance manoeuvrability (Webb 1994), other studies conclude conversely. For example, Hammerschlag et al. (2018) in a study with seven teleost prey species, showed a negative correlation between abundance of predator and morphological traits associated with predator detection and evasion such as caudal fin size and eyes size, which were smaller in habitats with high presence of predators. In addition, Langerhans et al. (2004) showed morphological differences in *Gambusia affinis* related to the presence of predators. Specifically, they found that fishes in environments with predators had larger caudal peduncles, smaller heads and more elongated bodies. Some of these results are in concordance with ours, since the wild population which was exposed to predators, displayed more fusiform body than captive population.

The negative correlation of some morphological traits (axis 1 of the DFA) with MMR indicated that wild males and females displayed narrow and elongated bodies, which means a more fusiform body that it is known it favours higher MMR (Killen et al. 2010; Killen et al. 2016; Baktoft et al. 2016). In addition, some other morphological traits such as longer and narrower caudal fin, which are less expensive to drag (Rouleau et al., 2010), were positively correlated with SMR for wild males and females, then it seems obvious that differences in environmental conditions between wild and captive habitats could explain morphological and metabolic changes in these populations as a result of different environmental pressures through phenotypic plasticity.

As we know, this is the first study that showed metabolic and morphological phenotypic plasticity between wild and captive populations of Iberian toothcarp, suggesting that captive breeding conditions caused physiological and morphological changes by means of phenotypic plasticity, which could affect the success of reintroductions into the wild of this endangered species.

7. GENERAL DISCUSSION

In the Iberian Peninsula, the acclimatization of invasive species is one of the most important negative factors that affect the survival of endemic species (Elvira 1995). Numerous studies have summarized the impacts of invasive species on native species and the structure of the communities (Williamson 1996; Wilcove et al. 1998; Parker et al. 1999; Sala et al. 2000; Stein et al. 2000), and the effects on the ecosystems (Vitousek and Walker 1989). Ecological responses, such as dietary traits, growth and reproduction, usually show wide variability in non-native species when invading new habitats, with this being particularly clear in freshwater fishes (Stearns and Koella 1986; Fox and Crivelli 2001; Copp et al. 2005; Almeida et al. 2012; Tarkan et al. 2012). In this context, several studies have assessed the adaptation of fish to environmental changes through phenotypic plasticity (Kekäläinen et al. 2010; Oomen and Hutchings 2015; Oufiero and Whitlow 2016).

In addition several studies have demonstrated that captive breeding could affect morphology and physiology of fishes (Gross 1998; Saraiva and Pompeu 2016) and some of these studies have shown that these morphological and physiological changes reduce the survival of the individuals raised in captivity when they are released in the wild (Weiss and Schmutz 1999; Araki et al. 2007).

Our research has particularly focused on assessing the phenotypic plasticity of the invasive species *A. alburnus* which could explain its adaptability to new environments in Iberian freshwaters (chapters 4 and 5), and if the endangered fish species *A. iberus* showed phenotypic plasticity in morphological and physiological traits when comparing wild and captivity reared populations (chapter 6). Specifically, we hypothesized i) populations of *A. alburnus* that came from more variable and unpredictable environments (internal basins of Catalonia, chapter 4) would present a more opportunistic vital strategy such as smaller size at maturity, higher reproductive investment, smaller size, lower growth index, lower body condition, early age at maturity and more variability in its diet composition than populations that live in more stable and predictable environmental conditions (rivers of the rest of the Iberian Peninsula and native population, chapter 5); ii) the native population of *A. alburnus* would present a more equilibrium vital strategy such as larger size at maturity, lower reproductive investment, larger size, higher growth index, higher body condition, later age at maturity and less variability in its diet composition than populations introduced in different rivers of the Iberian Peninsula (chapters 4 and 5); iii) swimming capacity, metabolism and morphology would be different between wild and captive reared populations of *A. iberus* (chapter 6).

First, the assessed life-history traits showed highly variability between the study rivers, suggesting wide phenotypic plasticity in bleak. Specifically, *A. alburnus* populations that came from rivers with more variable and unpredictable environmental conditions (internal basins of Catalonia, chapter 4), showed lower growth rates, high reproductive investment, earlier age at maturity, smaller size at maturity and more variability in dietary traits than *A. alburnus* populations that came from large rivers of the Iberian Peninsula and native population characterized by less variable and more predictable environmental conditions (chapter 5) overall. Therefore, these results confirm our first hypothesis since *A. alburnus* populations came from more variable and unpredictable environmental conditions (chapter 4) showed a more opportunistic strategy than other studied populations.

Second, native *A. alburnus* population showed the values of growth, reproduction and dietary traits that are more characterized with equilibrium strategy in the different studied populations (chapter 4 and 5). The values of age at maturity and length at maturity were the highest, body condition showed the second highest value and reproductive traits such as fecundity were the lowest. Furthermore, native population showed the highest values of zooplankton in its diet composition, showing that populations of *A. alburnus* in native areas prefer to consume these type of food (Politou 1993) and a clear adaptation to a food availability when this species invading new areas (chapter 4 and 5). Therefore, our results of life-history and dietary traits in native population were more related to equilibrium strategy which are representative of more stable and predictable environmental conditions (Mims and Olden 2012). Hence, these results confirm our second hypothesis.

Third, high variability in dietary traits were found between different invasive *A. alburnus* populations studied in Iberian Peninsula whereas native population showed less variability in its diet composition. Bleak mainly inhabits still-waters in its native area, this species is well-adapted to play an ecological role as an open-water feeder, with diet being chiefly based on zooplankton (Vinni et al., 2000; Vašek and Kubečka 2004). Our results, showed that bleak can modulate this zooplanktivorous strategy at each particular habitat patch to increase resource partitioning and consequently, reduce competition with co-existing fish species. Therefore, our findings indicate an elevated capacity of this fish to use food resources apparently less suitable to its morphological adaptations (e.g. conspicuous superior mouth). In overall, these results suggest that bleak could deeply change the foraging strategy, from a ‘pelagic’ to a ‘benthic’ feeder, which may aid bleak to better thrive at each Iberian river, where zooplankton availability may be a more limited trophic resource (Almeida et al. 2017). Accordingly, dietary traits were clearly variable among the study rivers, indicating wide plasticity in bleak foraging strategies dependent on particular river conditions (i.e. habitat heterogeneity, food supply). Therefore, our second hypothesis in order to dietary traits, also was confirmed.

Fourth, although differences in water flow can induce morphological differences between fishes of the same species and this entails an adaptation of the swimming capacities related to the environmental condition (Fu et al. 2013; Senay et al. 2017), our results showed no differences in swimming capacity between captive and wild populations (chapter 6). These results may be showing that *A. iberus* is a species that prefer to inhabit in slow water flow habitats (Vargas Pera 1993; Alcaraz et al. 2008), and this type of habitats not differed excessively of captive one (chapter 6). Further, we found differences in physiologycal traits between *A. iberus* populations (chapter 6). Wild population showed higher SMR, MMR and AAS than captive population. These results suggest that environmental factors related to each type of habitat where populations came from could explain the differences found. Specifically, we suggest that differences in SMR could be related to food availability (Auer et al. 2015), energy intake history (Rosenfeld et al. 2015) and presences of predators (Fu et al. 2015; Auer et al. 2018). MMR differences found between populations suggest that concentration of water salinity (Prakoso et al. 2018; Dalziel et al. 2012), food search and predator avoidance (Fu et al. 2015; Metcalfe et al. 2016) could be the reasons to explain these differences. Regards to AAS, environmental factors such as predator-prey interactions, hydrologic alterations (Farrell 2016) and food availability (Auer et al. 2015), could explain differences found between populations. All these results partially agree with our third hypothesis.

Fifth, several studies have showed that captive breeding conditions can induce morphological changes in body shape, head and fins' proportions (Swain et al. 1991; Fleming et al. 1994; Hard et al. 2000; Solem et al. 2006; Belk et al. 2008). In our study (chapter 6) high variability of morphological differences were found between captive and wild populations and between sexes of Iberian toothcarp. Shortened and deeper caudal peduncle may indicate a greater capacity to accelerate at the expenses of longer steady swimming and to enhance manoeuvrability (Webb 1994), typical of more complex habitats and related to avoid predators. Conversely, in our results, wild population, which came from of an environment more complex and with presence of predators, showed a more fusiform morphology with narrower heads, narrower bodies and narrower and longer caudal peduncles than captive population overall. For example, Saraiva and Pompeu (2016), showed differences between captive and wild populations *Prochilodus lineatus*. Specifically, they found a higher body height and wider caudal fin in wild populations which related to the need to perform manoeuvres to avoid obstacles and swim longer because they lived in a larger territory than captive one. Similar results were found by Olsson and Eklöv (2005), they showed morphological differences in two populations of *Perca fluviatilis* that they related to habitat complexity and prey type diversity. Further, Brönmark and Miner (1992), found a positive correlation between body width in individuals of *Carassius carassius* and presence of predator *Esox lucius*. Therefore, our results do not agree with several studies carried out to date with other species, although other studies do agree in part with our results. Similar results to ours were found by Garduño-Paz et al. (2010), they showed morphological differences related to the complexity of the environment among populations of *Gasterosteus aculeatus*. Specifically, like some results of our study, they found that fish that came from more complex environments, in which there were rocks and plants, had more fusiform bodies and smaller heads. They suggest that a more hydrodynamic body facilitates swimming between rocks and plants and a smaller head can help to catch prey in the interstitial spaces. Similarly, Black et al. (2017), showed differences in the position of the head between captive and wild populations of *Cyprinodon bovinus*. They showed that captive population had upward repositioning of the head that they related to the less complex environment. Similar findings also were reported by Wilcox and Martin (2006) with *Cyprinodon diabolis*, they compared native and captive populations and, like us, they showed that captive population had deeper bodies and upward position of the head than wild. Morphological differences between sexes were more relevant between captive females that showed deep bodies and wide caudal peduncles and wild males, which showed fusiform bodies and long caudal peduncles. Although all these results confirm our third hypothesis, differences found between populations and sexes related to specific environmental factors of each habitat, lead us to ponder on the need to continue researching in this field with more specific experiments in order to more reliably demonstrate the morphological differences found.

Sixth, some morphological features were correlated with physiological traits (chapter 6). In our results, we found that wild population which displayed narrow and elongate bodies were correlated to MMR and SMR (Table 6.4) and showing a relationship between a more fusiform body and higher MMR and SMR. Specifically to MMR our results suggest that environmental factors related to wild environment such as presence of predators, water salinity concentration and ability to catch prey would affect morphology and entail~~s~~ these changes could be related to higher MMR (Killen et al. 2010; Killen et al. 2016; Metcalfe et al. 2016, Baktoft et al. 2016; Prakoso et al. 2018). We do not rule out that food availability may also increase MMR rates although this latter consideration should be demonstrated with more specific studies. In order to SMR, we also suggest that our results could be related to environmental factors such as food availability, presence of predators and energy intake history (Norin and Malte 2011; Fu et al. 2015; Rosenfeld et al. 2015; Liu et al. 2016), that could be affected by the morphological features described in wild population.

Seventh, in order to avoid differences between wild and captive populations, we suggest the following recommendations to those responsible for carrying out captive breeding projects of *A. iberus:* 1) maintenance of the physicochemical characteristics of water in relation to the natural environment (eg salinity, temperature); 2) complexity of habitat regarding the composition and structure of aquatic vegetation; 3) sex-ratio; 4) density of individuals; 5) type and availability of food similar to that of its natural habitat 6) presence of predators.

Finally, the results of this doctoral thesis evidence the important role that play different environmental conditions to induce changes in life-history traits such as growth, reproduction and dietary traits in invasive *A. alburnus* in Iberian Peninsula freshwaters and how it improves the adaptation of different populations studied in chapter 4 and 5, which were subjected to different environmental conditions (Stearns 1983; Stearns 1989; Scheiner 1993; Agrawal 2001; Hoverman and Relyea 2008). On the other hand our results also showed that environmental factors related to captive and wild environments such as habitat complexity, food availability, presence of predators, salinity, inter-intraspecific competition, food search and energy food intake history could induce physiological and morphological variability among *A. iberus* populations through phenotypic plasticity (Svanbäck 2004; Jonsson and Jonsson 2006; Eklöv and Jonsson 2007; Almeida et al. 2008; Saraiva and Pompeu 2014; Metcalfe et al. 2016; Saraiva and Pompeu 2016; Liu and Fu 2018) but it did not show any significant influence in *Ucrit* between studied populations.

8. GENERAL CONCLUSIONS

The main conclusions of this thesis are:

1. High variability in life-history traits such as growth, reproduction and diet have been demonstrated in *A. alburnus* populations inhabiting Iberian freshwaters.

2. Invasive Iberian *A. alburnus* populations that came from more variable and unpredictable environments showed values of growth, reproduction and dietary traits more typical of the opportunistic strategy.

3. Invasive Iberian *A. alburnus* populations that came from more stable and predictable environments showed values of growth reproduction and dietary traits more related to equilibrium vital strategy.

4. Native *A. alburnus* population displayed life-history and dietary traits typical of equilibrium strategy.

5. Phenotypic plasticity may facilitate the spread and establishment of *A. alburnus* in new non-native areas.

6. No differences in swimming capacity between captive and wild *A. iberus* populations have been found.

7. Differences in metabolic traits and morphology have been demonstrated in the studied *A. iberus* populations.

8. Significant differences have been found in morphology and metabolic traits between captive and wild *A. iberus* populations. Specifically, wild population displayed higher SMR, RMR and MMR and more fusiform bodies than captive one.

9. Wild population which came from a more complex habitat and where there was presence of predators showed larger caudal peduncles, smaller heads and more fusiform bodies in comparison to captive one which came from a habitat structurally less complex and without predators.

10. Morphological features of wild *A. iberus* population such as more fusiform bodies and longer and narrower caudal peduncles have been correlated to higher SMR and MMR.

11. Morphological features of captive breeding *A. iberus* population such as wider bodies, shorter and wider caudal peduncle, and larger heads have been related to lower SMR and MMR.

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10. ANNEX

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Inter-population plasticity in growth and reproduction of invasive bleak, *Alburnus alburnus* (Cyprinidae, Actinopterygii), in northeastern Iberian Peninsula

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Abstract. The bleak, *Alburnus alburnus*, is an invasive fish in the Iberian Peninsula, where this species mainly disturbs the highly endemic fauna via competition and aggression. Despite this impact, information on bleak autecology is scarce in the Iberian Peninsula, with no data on growth and reproduction. The aim of the present study was to compare bleak populations across four Iberian streams: Muga, Fluvià, Cardener and Foix (northeastern Iberian Peninsula). These streams have similar environmental conditions at the regional scale (e.g. Mediterranean climate, geomorphology). In Muga and Foix streams, bleak showed lower growth rate and back-calculated length at age 2. Body condition was lower in Foix streams, whereas length at maturity was higher. In Muga stream, the proportion of females was lower. In Cardener stream, bleak showed higher back-calculated lengths at ages 1 and 2, growth rate, body condition and reproductive investment. Results showed that bleak populations are able to display wide phenotypic plasticity in small Mediterranean-type rivers. Specifically, bleak population "health" appears to be better in Cardener stream, whereas it is worse in Muga and Foix streams. Present findings suggest that inter-population plasticity allows bleak more successfully to invade Mediterranean fresh waters in the Iberian Peninsula.

Key words: back-calculation, body condition, non-native fish, sex-ratio, sexual maturity

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Introduction

One of the more relatively recent arrivals in the Iberian Peninsula is the bleak *Alburnus alburnus* (L., 1758), a cyprinid species native to most of Europe, from the Pyrenees to the Urals. In the native area, bleak inhabit lakes or still waters in medium-large rivers and feed chiefly on zooplankton (Freyhof & Kottelat 2008). In the Iberian Peninsula, this species was mainly introduced in reservoirs during the 1990s as a "forage fish" for non-native piscivores such as northern pike *Esox lucius* L., 1758, largemouth bass *Micropterus salmoides* (Lacépède, 1802) or pikeperch *Sander lucioperca* (L., 1758) (Vinyoles et al. 2007). Since its introduction, the bleak has displayed a strong invasive character throughout Iberian fresh waters and it is potentially dangerous for the highly endemic fish fauna, mainly via competition and aggression (Leunda 2010). However, the only information available on this species in the Iberian Peninsula simply addresses its distribution (Vinyoles et al. 2007) or size structure (Almeida et al. 2014).

In particular, growth and reproduction are important traits of fish life-history, which can show wide phenotypic plasticity under contrasting local conditions (e.g. Fox & Crivelli 2001, Tarkan et al. 2010). However, no data on growth or reproduction of invasive bleak is known to exist for the Iberian Peninsula. Furthermore, studies of bleak autecology in this region are mainly focused on reservoirs, with few data from water courses (Almeida et al. 2014). Therefore, information in the present paper is highly relevant to understand invasion features of this fish species in the Mediterranean region of Europe.

Consequently, the aim of the present study was to assess the inter-population plasticity of bleak under variable local conditions in Mediterranean fresh

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waters. For this purpose, bleak populations were compared across selected Iberian streams showing similar environmental conditions at the regional scale. Specifically, the following population parameters were examined: back-calculated length at ages, growth rate, body condition, reproductive investment, length/age at maturity and sex-ratio.

Material and Methods

Study area

Bleak populations were sampled in four small water courses (i.e. streams, < 100 km river length), that are located at < 250 m a.s.l. in Catalonia (northeastern Iberian Peninsula) and drain into the Mediterranean Sea (from North to South): Muga, Fluvià, Cardener and Foix. These study streams were selected because they are geographically in close proximity to each other (latitude = 41°19′-42°16′ N, maximum distance ≈120 km) and, at the regional scale, possess similar fish assemblages, limnology and geomorphology, i.e. bed shape, wetted width, flow, substratum composition, riparian vegetation and level of human disturbances (see details in Catalan Water Agency 2015). Bleak abundances were also similar between study streams (CPUE = 7.6-9.6 ind. 100 m^{-2}). All of these conditions allow that variations among bleak populations are more likely to be attributable to environmental factors operating at the local scale (i.e. within stream), such as food supply or habitat availability. Furthermore, the study streams show a typical Mediterranean hydrological regime (i.e. autumn-winter floods and summer droughts) and they can be used as reference systems for assessing the ecological responses of this invasive species where introduced to other fresh waters in the Mediterranean region of Europe (Almeida et al. 2014). A final key point for selection of these water courses is that bleak were introduced in the four streams around the year 2000 and thus, their populations are currently well established and spreading in these habitats (Vinyoles et al. 2007). Consequently, the study populations are at the same ”invasion stage” and therefore, this potential effect on the assessed parameters was controlled.

Field sampling

To achieve an accurate assessment of bleak reproductive traits and to avoid any temporal bias across the study streams, fish were collected for two weeks from late May to early June 2012, just before the spawning period of this species in the study area. Bleak were collected along 10 km river (n = 10 sampling sites, one site per km) in the middle reach of each stream by electrofishing (2000 W pulse DC generator at 200-250 V, 2-3 A) and dip nets (1.5 m long pole, 30 cm diameter net, 10 mm mesh size). The sampling method consisted of following a zigzaging and upstream direction at each site (50 m river long, 30 min). To encompass the existing environmental variability, fish were collected from all meso-habitats present in the study streams (i.e. runs, riffles and pools), thus obtaining a representative sample of bleak across the broadest possible body size range from every stream.

After each survey was concluded, to analyse comparable samples of similar number, 80-90 undamaged bleak individuals were stored from each study stream. They were immediately immersed in an overdose solution of anaesthetic (MS-222) for 15 min followed by severance of the spinal cord. Finally, bleak individuals (n = 336: 84, 88, 83 and 81 from Muga, Fluvià, Cardener and Foix streams, respectively) were stored in ice during transport to the laboratory.

Laboratory procedures

On the arrival at the laboratory, bleak were measured for total length (TL, ±1 mm) and eviscerated weight (We, ±0.01 g). Also, scale samples were taken from the area between the lateral line and dorsal fin. Then, fish were dissected to determine gender and maturity status in females, which were classified as sexually mature if their ovaries contained yolked eggs (e.g. Tarkan et al. 2009). Reproductive parameters (except for sex-ratio) were analysed in mature females only, as this “fraction” of fish population is the most relevant for the assessment of future viability and subsequent invasiveness in small Mediterranean-type rivers (Vila-Gispert et al. 2005). Therefore, mature females were also measured for gonad weight (Wg, ±0.1 mg) by using an electronic balance.

Age was determined by counting true annuli from acetate impressions of scales, and read on micro-projector (magnification: 48×). Age determinations were completed independently by two readers and when the interpretations were different, an additional reading was made. If the disagreement continued, then the sample was excluded from the analyses. The total scale radius and radius of annual increments were measured from the focus to the posterior edge along the anterior-posterior axis.

Data analyses

As linear equation gave a better fit than non-linear equation for the relationship between scale radius and body length, back-calculation of TL at ages were estimated by the Fraser-Lee equation (Francis 1990): $L_t = c + (TL_c - c)(S_t/R)$, where L_t is TL when growth mark t was formed, TL_c is TL at the time of capture, S_t is the distance from scale centre to the growth mark t, R is the scale radius, and c is the intercept on the length axis from linear regression between TL and scale radius. To reduce bias due to size differences in the size distribution of the examined populations, "c" value was accepted as 12 mm and used as fixed body length-scale intercept.

For comparisons of growth trajectories, Hickley & Dexter (1979) procedure was followed: 1) mean TL at age n were plotted against TL at age (n + 1) to obtain a straight line for the Walford (1946) method; 2) TLs at age were obtained from the formula $l_n = L_\infty (1 - k^n)$, where l_n = TL at age n, $L_\infty = l_t/(1 - k)$, l_t = interception on the y axis and k = slope of the Walford plot (Hickley & Dexter 1979); 3) TLs at age for each year class were expressed as a proportion (%) of the TLs from the Walford method; 4) these proportions were used to calculate a mean growth index (GI) and thus to estimate relative growth of each population.

To provide an integrated impression of true body condition (BC), eviscerated weight (We) was used to avoid bias from the weight of gonads and gut contents. To assess reproductive investment (RI) in mature females, Wg was used (e.g. Almeida et al. 2014).

Age at maturity (AaM) of each population was calculated from the percentage of mature females in each age-class using the DeMaster (1978) formula as adapted by Fox (1994):

$$\alpha = \sum_{x=0}^{w} (x)\,[f(x) - f(x-1)]$$

where α is the mean AaM, x is the age in years, $f(x)$ is the proportion of fish mature at age x, and w is the maximum age in the sample. A modified version of this formula (10 mm TL intervals in place of age-classes) was used to calculate mean length at maturity (LaM) as per Fox & Crivelli (2001).

Previous analyses (i.e. GLMs) did not find differences between males and females for the assessed parameters on growth (i.e. back-calculations, GI, BC) and consequently, this factor (i.e. gender) was not included in subsequent statistical approaches. Differences in mean back-calculated TLs were analysed by using one-way analysis of variance (ANOVA) for repeated measurements. One-way ANOVA was used to test for significant differences of GI between populations. One-way analysis of covariance (ANCOVA) was used to test for significant differences of BC (covariate: TL) and RI (covariate: We). ANOVAs and ANCOVAs were followed by a *post hoc* Tukey-Kramer honestly significant difference (HSD) test. Male-to-female ratio was tested using the chi-squared (χ^2) test. Data were transformed by using ln (x + 1). Particularly for percentage data, logit-transformation was used. Assumptions of normality of distributions and homogeneity of variances were verified through Shapiro-Wilks and Levene's tests, respectively. All statistical analyses were performed with SPSS v.19 (SYSTAT Software Inc., Chicago, U.S.A.). The significance level was set at α = 0.05.

Results

Bleak individuals ranged from 43 to 144 mm TL. Significant growth differences were detected among back-calculated TLs at age 1 in the four examined populations ($F_{3,262} = 36.06$, $P < 0.001$), with bleak from Cardener stream showing the highest mean back-calculated TL and Muga population showing the lowest mean TL. Bleak populations in Foix and Fluvià streams made a different significant group for TL at age 1 (Table 1). Differences were also found for the back-calculated TL at age 2 ($F_{3,101} = 21.66$, $P < 0.001$), with Cardener and Fluvià populations showing the greatest mean TLs, whereas Muga and Foix populations showed the lowest mean values (Table 1). Age 3 was only found in Foix and Fluvià populations, with the latter stream showing the highest TL (Table 1). Significant differences were found between populations for the GI ($F_{3,317} = 4.83$,

Table 1. Population parameters of bleak *Alburnus alburnus* for every study stream: back-calculated total length (TL, mm) at ages (A_n), growth index (GI, %), body condition (BC, We in g), reproductive investment (RI, Wg in g), TL at maturity (LaM, mm), age at maturity (AaM, years) and sex-ratio (male ÷ female). Results are means (A_n, GI)/adjusted means (BC, RI) ± SE. Significant differences between bleak populations are shown by superscripts, after Tukey HSD and χ^2 tests ($P < 0.05$).

Study stream	A_1	A_2	A_3	GI	BC	RI	LaM	AaM	Sex-ratio
Muga	$^{a}47.9 \pm 5.7$	$^{a}86.2 \pm 5.7$	-	$^{a}86.2 \pm 6.5$	$^{a}8.75 \pm 0.24$	$^{a}1.29 \pm 0.24$	79.8	0.91	$^{a}2.86$
Fluvià	$^{b}58.1 \pm 5.6$	$^{b}98.5 \pm 4.1$	125.9 ± 6.4	$^{b}106.9 \pm 6.7$	$^{b}7.76 \pm 0.22$	$^{a}1.11 \pm 0.23$	79.1	0.86	$^{b}1.36$
Cardener	$^{c}75.0 \pm 2.5$	$^{b}100.3 \pm 4.7$	-	$^{b}116.1 \pm 6.4$	$^{c}11.02 \pm 0.19$	$^{b}2.33 \pm 0.20$	87.0	1.14	$^{b}1.64$
Foix	$^{b}60.3 \pm 6.1$	$^{a}79.3 \pm 7.2$	[1]86.8	$^{a}88.9 \pm 5.0$	$^{b}7.43 \pm 0.20$	$^{a}1.44 \pm 0.21$	92.0	1.00	$^{b}1.97$

[1] Only one A_3 specimen was collected in Foix stream.

$P < 0.01$), which showed that the fastest bleak growth was in Cardener and Fluvià streams, and then Foix and Muga populations (Table 1). The highest and the lowest BC were observed in Cardener and Foix/Fluvià streams, respectively, with significant differences between populations ($F_{3,316} = 70.08$, $P < 0.001$). Bleak from Muga stream made a third group intermediate between Cardener and Foix/Fluvià streams (Table 1).

Regarding reproductive parameters, significant differences were found between populations for RI ($F_{3,93} = 3.25$, $P < 0.05$), with the highest adjusted Wg value in Cardener population, followed by a second group made by Foix, Muga and Fluvià streams (Table 1). The highest mean LaM was found in Foix stream, then Cardener and both Muga and Fluvià populations showed the lowest values (< 80 mm LaM) (Table 1). The youngest mean AaM was found in Fluvià stream, whereas the oldest mean for this parameter was found in Cardener stream, with both Foix and Muga populations showing intermediate values (Table 1). All bleak populations were dominated by males, with the Muga population possessing the highest sex-ratio ($\chi^2_3 = 28.94$, $P < 0.05$), followed by Foix, Cardener and Fluvià streams (Table 1).

Discussion

Life-history traits are expected to change in animal populations in response to variations in biotic and abiotic conditions at the local scale (Stearns & Koella 1986), with this being particularly clear in freshwater fishes (Fox & Crivelli 2001). These changes also apply to non-native species when invading new habitats (e.g. Tarkan et al. 2012), where they usually display high phenotypic plasticity and thus contribute to a more successful invasion process (Agrawal 2001). In particular, Mediterranean-type rivers typically show a high hydrological variability, including autumn-winter floods and summer droughts, which may promote great differences in local conditions between contiguous catchments (Boix et al. 2010). Accordingly, the population parameters examined in the present study were highly variable between the study streams, suggesting wide phenotypic plasticity in bleak. Specifically, bleak population "health" appears to be better in Cardener stream, where growth, body condition and reproduction showed the fastest rate, best status and highest investment, respectively. On the contrary, bleak populations showed overall lower growth rate, body condition, reproductive investment and proportion of females in Muga and Foix streams. The ability of this invasive fish to overcome changes in local conditions has been also shown elsewhere in the Iberian Peninsula by Almeida et al. (2014), regarding size structure between contrasting habitats (river *versus* reservoir). Beside this, the present findings on growth and reproduction also suggest that the wide inter-population plasticity displayed by non-native bleak may be a mechanism for this species more successfully to invade novel Mediterranean freshwater ecosystems.

The observed LaM and AaM in the study streams were lower than in native populations under similar climate conditions (e.g. > 100 mm TL and ≈2 years, see Politou 1993). These two traits (i.e. low length and age at maturity) are typical in populations that are in an expansion stage, facilitating the subsequent bio-invasion (Bohn et al. 2004). Studies with a variety of fish species, including non-native populations, have demonstrated that patterns of body development vary in relation to abiotic and biotic factors operating at the local scale such as temperature, competition or food availability (e.g. Tarkan et al. 2010, 2012). These findings also support the hypothesis that bleak could change their growth and reproductive traits under particular conditions within every study habitat (Almeida et al. 2014). Thus, non-native species may switch their life-history strategies at the initial stage of invasion when they are introduced in novel ecosystems and confronted with variable environmental scenarios (Ribeiro & Collares-Pereira 2010, Tarkan et al. 2012). This might be related to epigenetic mechanisms in which some progeny of invaders show a better survival rate than those of species in its native range. Such phenotypic plasticity has been shown for several non-native fish species belonging to a variety of taxonomic families: Gobiidae such as bighead goby *Ponticola kessleri* (Günther, 1861) and round goby *Neogobius melanostomus* (Pallas, 1814) (L'avrinčíková & Kováč 2007, Kováč et al. 2009), Cyprinidae such as topmouth gudgeon *Pseudorasbora parva* (Temminck & Schlegel, 1846) and gibel carp *Carassius gibelio* (Bloch, 1782) (Záhorská & Kováč 2009, Tarkan et al. 2012) or Centrarchidae such as pumpkinseed *Lepomis gibbosus* (L., 1758) and largemouth bass (Ribeiro & Collares-Pereira 2010).

The present paper represents one of the few studies on autecology of invasive bleak in the Iberian Peninsula (see other two examples in Vinyoles et al. 2007 and Almeida et al. 2014), particularly providing insights into the population responses of this fish species to environmental conditions at the local scale. Nevertheless, the short period of this sampling (< 1 month) only reveals a snapshot of the study populations and consequently, there is the need for monitoring inter-annual variations of bleak traits. Moreover, the effect of specific environmental conditions on bleak populations is another key factor to be analysed in subsequent years. These long-term studies will provide with more accurate assessments of bleak status in Iberian fresh waters.

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