

## Inter-population variability in growth and reproduction of invasive bleak *Alburnus alburnus* (Linnaeus, 1758) across the Iberian Peninsula

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**Abstract.** The native European freshwater cyprinid fish, common bleak *Alburnus alburnus* (Linnaeus, 1758), is an invasive non-native fish in the Iberian Peninsula, where it threatens the valuable endemic fish fauna. Despite the bleak's invasive status, there is a dearth of studies on the biological traits of non-native populations in Europe's Mediterranean region, and the present study aimed to compare bleak growth and reproductive traits across the principal rivers of Iberia with those from a native 'reference' bleak population in France. Non-native bleak from the River Tagus had the highest back-calculated total lengths (TLs), growth rate and body condition, but the lowest reproductive investment and smallest egg size of all studied populations. Whereas, these latter two traits were the highest in the River Ebro, where fecundity was the lowest for all Iberian rivers. The youngest age and the smallest TL at maturity were observed in the River Ebro. The River Segura had the lowest back-calculated TLs and growth rate, the highest fecundity and proportion of females, and the oldest age at maturity. Population traits of bleak in the River Saône (the native 'reference' population) were generally different from those in Iberian rivers, except for the River Ebro. This wide inter-population variability contributes to the species' successful establishment in novel habitats.

**Additional keywords:** back-calculation, body condition, freshwater fish, non-native species, sex ratio.

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## Introduction

Invasive fishes can be major drivers of biodiversity loss in freshwater ecosystems at the global scale (Mooney and Hobbs 2000). The potentially disruptive effects of non-native fishes are of particular conservation concern in the Iberian Peninsula, where endemism in freshwater fishes is high (>50% of native species) and many of the endemic species are seriously threatened (Reyjol et al. 2007). Indeed, introduced fishes currently represent >30% of the total fish species and this continues to increase, which renders the Iberian Peninsula a hotspot for fish invasions (Leunda 2010; Almeida et al. 2013).

The common bleak *Alburnus alburnus* (Linnaeus, 1758) is native to most of Europe, from the Pyrenees Mountains to the Ural Mountains, and including the south-eastern part of Great Britain, where this cyprinid species inhabits lakes and medium-to-large rivers (Keith et al. 2011). In the Iberian Peninsula, reservoirs are important locations for the introduction of non-native fishes, such as the following trophy species: northern pike *Esox lucius*, largemouth bass *Micropterus salmoides*, Eurasian perch *Perca fluviatilis*, European catfish (also known as sheatfish) *Silurus glanis* and pikeperch *Sander lucioperca*. Common bleak was first introduced to Spanish reservoirs from southern France during the 1990s as a 'forage fish' for the above-mentioned piscivorous fishes (Vinyoles et al. 2007), including the rivers Ebro (in 1992), Guadiana (in 1999), Segura (in 2004) (Andreu-Soler et al. 2004), Tagus (in 2005) and Guadalquivir (in 2006). Since its introduction, the bleak has displayed a strong invasive character throughout Iberian fresh waters by natural and human-assisted (mainly stocking) dispersal, becoming abundant within Iberian fish assemblages (e.g. Maceda-Veiga et al. 2010). Nevertheless, Iberian fishes are poorly adapted to sustain the many introductions of non-native fishes, which usually results in native species being displaced from available resources. In particular, bleak threatens several Iberian fishes, mainly through trophic and interference (i.e. aggression) competition (Leunda 2010). As an example, direct observations of bleak by snorkelling have clearly shown that bleak can disturb the foraging behaviour of endemic Ebro nase *Parachondrostoma miegii* (Almeida and Grossman 2012).

From a conservation perspective, there is a clear need for information on bleak population traits across the Iberian Peninsula with which to assess the risks posed by this species to native species and ecosystems (Almeida et al. 2013). In particular, growth and reproduction are key aspects for assessing the phenotypic variability of non-native fishes, which can facilitate the invasion process (e.g. Copp and Fox 2007). However, variations in bleak population traits have received relatively little study across Europe in general and in the Mediterranean region in particular. The available literature on bleak autoecology are simply related to species' spatial distribution (Vinyoles et al. 2007), habitat preferences (Muñoz-Mas et al. 2016) and a few population traits restricted to local scales (Almeida et al. 2014; Masó et al. 2016).

The aim of the present study was to assess the inter-population variability of traits invasive bleak across the Iberian Peninsula, including back-calculated total lengths (TLs) at age, growth rate, body condition, reproductive investment, fecundity, egg size, age and TL at maturity and sex ratio. For this purpose, bleak populations were compared across the principal Iberian rivers

as well as with regard to a native bleak population of the River Saône (eastern France), which was used as the 'reference' population because France has historically been the 'donor region' for non-native fish introductions to Iberia along the so-called 'Perpignan–Barcelona corridor' (see Clavero and García-Berthou 2006).

## Materials and methods

Each Iberian sampling site was surveyed for two consecutive days by different methods (see details below). In particular, sampling dates were on 25–28 May for Ebro (two sites), 28–31 May for Tagus (two sites), 29 May–1 June for Guadiana (two sites), 22–25 May for Segura (two sites) and 7–10 May for Guadalquivir (two sites) and 5–6 June for Saône (one site). The following five main Iberian rivers, which are all of typical Mediterranean climate, were sampled for bleak along a latitudinal gradient (from north to south): Ebro (41°47'30"N, 1°05'24"W and 41°26'39"N, 0°28'26"W), Tagus (39°49'07"N, 4°20'25"W and 39°58'12"N, 4°42'17"W), Guadiana (38°59'58"N, 5°51'52"W and 38°50'18"N, 6°13'53"W), Segura (38°06'05"N, 1°17'50"W and 38°04'54"N, 0°53'37"W) and Guadalquivir (37°37'01"N, 5°35'45"W and 37°30'46"N, 5°56'38"W). These geographic coordinates (given in upstream to downstream order) correspond to two sampling sites per river. The River Saône reference site (47°02'13"N, 5°06'52"E), where the climate is temperate oceanic, was then surveyed in early June.

To provide an appropriate assessment of reproductive traits, fish were collected from May to June 2012, just before the spawning period of bleak for each study area (authors, pers. obs.). Sampling sites ( $n = 11$ , two sites per Iberian river plus one on the River Saône) were selected to encompass similar environmental conditions. In particular, land use was mainly for agricultural exploitation, sites were located in well-regulated middle reaches of the main channel, and far from the influences of main tributaries and towns. Also, sites were separated >50 km within each Iberian 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, European Commission 2014). This allowed obtaining a representative sample of bleak across the broadest possible TL-size range from each river. Catch methods consisted of following a zig-zagging 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). Electrofishing was performed the first sampling day and nets were used the second day. All Iberian surveys followed the same sampling protocols (e.g. proportional effort in terms of people and time) to ensure comparability among the study rivers, with methods being agreed and applied by each research team per river). A professional fisherman sampled for bleak in France. To encompass the existing environmental variability, fish were collected from all meso-habitats present in the study rivers (e.g. runs, pools, shallows). Fish were stored on ice during transport to the laboratory, before being frozen (−20°C). All field procedures complied with animal use and care regulations of Europe.

Bleak were defrosted and measured for TL ( $\pm 1$  mm), scale samples taken from the dorsal area on both flanks, and then dissected and weighed for eviscerated mass (eW,  $\pm 0.01$  g). Sex was determined, as well as the maturity status in females, which were classified as sexually mature if their ovaries contained yolked oocytes (e.g. Tarkan *et al.* 2016). Reproductive traits shared by both sexes (i.e. gonad investment, age and TL at maturity) were analysed in mature females only (Copp and Fox 2007; Masó *et al.* 2016). Therefore, mature females were also weighed for gonad mass (gW,  $\pm 0.1$  mg). 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,  $\pm 0.01$  mm) by using a PC-based image analysis (Aphelion, ADCIS, Saint-Contest, France).

Age was determined from scales using a micro-projector (magnification  $48\times$ ) by counting true *annuli* from acetate impressions (Tarkan *et al.* 2016). The total scale radius and that of annual increments were measured from the focus to the posterior edge along the anterior–posterior axis. Age determinations were completed independently by two readers and when the interpretations were different, an additional reading was taken. Age determination was validated by opercula examination from sub-samples (10% of individuals per river) under a binocular microscope ( $40\times$ ).

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:

$$TL_A = TL_0 + (TL_C - TL_0) \times (R_A : R_C)$$

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 Masó *et al.* 2016 and Tarkan *et al.* 2016 for the same procedure). The particular  $TL_0$  value was 14 mm, which was used as a fixed TL–scale intercept. This is in accordance with Masó *et al.* (2016), where an intercept of 12 mm was found for four Iberian bleak populations in north-eastern Spain.

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)

$$eTL_A = TL_\infty \times (1 - S^A)$$

and

$$TL_\infty = TL_Y \div (1 - S)$$

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

$$FEC = gW \times D$$

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):

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

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).

General linear models (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 generalised, linear, mixed models (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 ( $\chi^2$ ) 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. Statistical analyses were performed with SPSS v19 (SYSTAT Software Inc., Chicago, IL, USA). The significance level was set at  $P = 0.05$ . Sequential Bonferroni corrections were performed for every set of multiple tests.

## Results and discussion

In total, 1200 bleak ( $n = 100$  individuals  $\times 2$  sites  $\times 5$  Iberian rivers + 200 individuals  $\times 1$  site from the River Saône) were

**Table 1. Population traits of bleak *Alburnus alburnus* in each study river (from north to south), including back-calculated total length (TL, mm) at Age  $i$  ( $A_i$ ), growth index (GI, %), body condition (BC, eviscerated weight (eW), 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)**

Reported values are means ( $A_i$ , GI) or adjusted means (BC, RI, FEC, ED)  $\pm$  s.e., 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  $\chi^2$  (only for SR) tests ( $P <$  critical  $P$ -value from Bonferroni correction)

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

collected and processed, the minimum and maximum TLs being 44 and 199 mm. Significant differences were found between the six study populations (Table 1) 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 the Tagus population differed significantly in TL at Age 4 ( $F_{5,189} = 12.47$ ,  $P < 0.001$ ). Significant differences were found among populations for GI ( $F_{5,1194} = 20.41$ ,  $P < 0.001$ ) and BC/eW ( $F_{5,1193} = 7.12$ ,  $P < 0.001$ ). Body mass ranged from 0.65 to 53.74 g. Among reproductive traits, ovary mass ranged from 0.22 to 6.69 g. Significant differences were found among populations for RI ( $F_{5,328} = 21.51$ ,  $P < 0.001$ ). Absolute FEC ranged from 732 to 12 380 eggs, with significant differences found among populations ( $F_{5,328} = 16.89$ ,  $P < 0.001$ ). Oocyte size ranged from 0.73 to 1.41 mm ED, again with significant differences found among populations ( $F_{5,328} = 14.34$ ,  $P < 0.001$ )

Bleak from the River Tagus had the fastest growth rate, highest body condition value, and the lowest gonad mass and egg size values. Whereas Ebro bleak has the highest values for mean gonad mass and mean egg diameter, but the lowest fecundity of all Iberian populations studied. Segura bleak had the lowest growth rate and the highest fecundity. 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).

The youngest and oldest mean AaMs were found in the rivers Ebro and Segura respectively, whereas the shortest and longest mean LaMs were observed in the river Ebro and Saône respectively (Table 1). All of the bleak populations were dominated by males, with SR being lower in the rivers Segura and Saône, whereas the rivers Guadalquivir, Tagus and Guadiana has the second highest SR values, and the River Ebro an intermediate value (Table 1).

Bleak from the rivers Saône and the closest Iberian river, the, had similar population responses (i.e. growth and reproductive traits), except for age and TL at maturity. This would seem to reflect the temperate oceanic (River Saône) v. Mediterranean climatic conditions under which these populations exist, the former being typical of the bleak's native range (Keith et al. 2011). The earlier sexual maturity in bleak under the warmer Mediterranean climate of the River Ebro is the same as observed between southern and more northerly populations of introduced pumpkinseed *Lepomis gibbosus* (Copp and Fox 2007; Cucherousset et al. 2009). A similar pattern has been observed in non-native populations of crucian carp *Carassius carassius* in England, where they benefit from 'benign' climatic conditions relative to other native populations in continental Europe (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 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 fresh waters, where a high abundance of both non-native and native piscivorous species co-exist (e.g. cormorants, herons, otters and invasive fishes). Moreover, the abundance of endemic cyprinid prey species (i.e. *Barbus*, *Chondrostoma*, *Leuciscus*) 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.

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, Serbia (Lujčić *et al.* 2013), and Pilica, 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 1), indicating a 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 1). In the Danube, Lujčić *et al.* (2013) also observed a higher sex ratio (2.27) than that found in the River Saône (Table 1). Bleak reproductive investment (GSI) the River Meuse, Belgium (Rinchar 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 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, which has been used as a surrogate for temperature regime (e.g. Cucherousset *et al.* 2009). Indeed, TL at maturity increases significantly ( $F_{5,7.68}$ ,  $P = 0.04$ ;  $LaM = 1.449Lat. + 30.664$ ,  $r^2 = 0.606$ ) with increasing latitude (data from Table 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).

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 (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. 37–47°N, against 47–59°N in Cucherousset *et al.* (2009) and 50–62°N in Tarkan *et al.* (2016) based on a greater number of study sites ( $n = 10$  and 20 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’.

The present paper 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. Topics for future research include food and 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).

## Conflicts of interest

The authors declare that they have no conflicts of interest.

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