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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

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 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.

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 , $L_\infty = l_1/(1 - k)$, l_1 = 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 $\alpha = 0.05$.

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 Fluvia 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 Fluvia 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 Fluvia 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 ± 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
Fluvia	^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.

$P < 0.01$), 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'avrinčiková & 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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