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Original Article

Effects of captive-breeding conditions on metabolic and performance traits in an endangered, endemic cyprinidontiform fish

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Abstract

Captive breeding and stocking are commonly employed strategies for enhancing fisheries and conserving endangered fish species. However, hatchery-raised fish often exhibit reduced performance in the wild, displaying alterations in physiological, morphological, and behavioral traits. We tested for differences in swimming capacity and metabolic traits between wild and hatchery-reared individuals of the Spanish toothcarp (*Aphanius iberus*) from 2 different populations. Furthermore, we experimentally tested if these changes translated into fitness differences after their stocking into the wild. There were significant differences in swimming capacity and metabolic traits between wild and hatchery-reared individuals and also between the 2 populations. Captive-bred individuals displayed consistently lower metabolic rates than wild individuals from the same population (30–76% lower). Critical swimming speed rather differed between the 2 populations. Sex-specific differences were observed in maximum and standard metabolic rates, with wild individuals and females generally exhibiting higher values but with some exceptions. During a 3-month experiment, survival rates did not significantly differ between wild and captive-bred fish. Captive-bred individuals started smaller but exhibited rapid growth during the experiment. Initially, larger captive-bred fish had lower body conditions than their wild counterparts, but these differences progressively diminished. In summary, captive-bred individuals of this fish species showed lower metabolic rates, although the differences with wild individuals slightly depended on sex and size.

Key words: fish stocking, hatchery-reared fish, metabolic traits, protected fish species, reintroduction, swimming capacity.

Captive breeding of threatened species is widely used to restore viable populations of native species within their former ranges and to enhance wild populations that are currently in decline (Seddon et al. 2007; Seddon, 2010; Crates et al. 2022). Although captive breeding and subsequent reintroduction are commonly used as a conservation tool, success rates of reintroductions are often low for several reasons (Black et al. 2017). For instance, natural habitats are generally very different from captive environments, and it has been demonstrated that captive individuals, when released in the natural environment, might have species fitness compromised due to changes in physiological, morphological, and behavioral traits (Kohane and Parsons 1988; Ruzzante and Doyle 1993; Lynch and O'Hely 2001; Wassink et al. 2022). Captive-reared individuals often have less capacity for quickly adapting to the natural habitat of their ancestors (Kleiman 1989; Fischer and Lindenmayer 2000; Seddon et al. 2007; Teixeira et al. 2007; Crates et al. 2022).

Studies comparing wild fishes and their captive conspecifics have demonstrated that fish raised in captivity can show differences in genetic structure (Schönhuth et al. 2003), morphology (Belk et al. 2008), behavior and survival (McGraw et al. 2002; Näslund 2021), and swimming capacity and metabolic traits (McDonald et al. 1998; Basaran et al. 2007; Wegner et al. 2018; Latorre et al. 2020). Therefore, unfavorable consequences associated with captive breeding have the potential to negatively impact the ability of captive individuals to survive, grow, or reproduce when released into the wild (Snyder et al. 1996; Woodworth et al. 2002; Fraser 2008; Crates et al. 2022; Wassink et al. 2022), thereby compromising conservation efforts (Einum and Fleming 2001; Wilke et al. 2015; Black et al. 2017; Berger-Tal et al. 2020; Crates et al. 2022). Moreover, numerous studies have consistently shown that the duration of time spent in a captive environment is directly correlated with a decline in mean population fitness (Lynch and O'Hely 2001; Ford 2002; Houde et al. 2010). Additionally, extended periods of captivity have been associated with an increased vulnerability to predator-induced mortality (Álvarez and Nicieza 2003; Huntingford 2004) and a diminished reproductive success (Araki et al. 2009). Hence, the ultimate survivorship, growth, and reproductive rates observed in reintroduced stocks play a pivotal role in determining the overall success of reintroductions into natural environments (Armstrong and Seddon 2008). Notably,

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these effects may be particularly pronounced in individuals who have experienced prolonged periods in captivity (Houde et al. 2010).

The Spanish toothcarp *Aphanius iberus* (Valenciennes 1846) is an endemic aphaniid species of the Iberian Peninsula and is listed as endangered by the International Union for Conservation of Nature and the Spanish legislation. It inhabits salt marshes and lagoons along the Mediterranean coast of Spain (Oliva-Paterna et al. 2006), although habitat loss and the presence of invasive species threatens the viability of remaining populations (García-Berthou and Moreno-Amich 1992; Rincón et al. 2002; Alcaraz et al. 2008). To improve the conservation status of the Spanish toothcarp, several breeding programs have been developed in the Iberian Peninsula by regional authorities, as an additional conservation tool.

Considering that changes in swimming capacity and metabolic traits could affect the performance and fitness of captive breeding populations, we asked whether hatchery-reared fish would be successful after being released into the wild. To address this question, we first tested for differences in swimming capacity and metabolic traits between wild and captive populations of the Spanish toothcarp and afterward, we experimentally tested if these changes translated into performance differences (i.e., survival, growth, and body condition), once they are reintroduced into the wild. We hypothesized that hatchery-reared fish, exhibiting potential alterations in these traits, may face challenges in successfully adapting to and thriving in the wild on release. By improving this knowledge, we can shed some light on captive breeding programs to enhance the management and conservation of this and other endangered fish species.

Materials and Methods

Experiment 1: respirometry trials and critical swimming speed

Sixteen males and 16 females of the Spanish toothcarp were collected in 2018 using a dip net from the hyperhaline coastal lagoon Clot de la Llúdriga (42° 15′ 34″ N, 3° 8′ 48″ E), hereafter named as "Population A wild," situated in the Alt Empordà wetlands, NE Iberian Peninsula (García-Berthou and Moreno-Amich 1992). Twenty-three males and 34 females were obtained from a stock in the University of Girona, the ancestors of which came from the same lagoon (hereafter, "Population *A* captive"). In addition, 15 males and 17 females were provided by the Ichthyological Centre of Ebro Delta (Ebro Delta Natural Park, Deltebre, Spain); the ancestors of these fish came from the wild population of Bassa del Fra Ramon lagoon (42° 01′ 49″ N, 3° 11′ 29″ E) (hereafter, "Population *B* captive") in the Baix Empordà wetlands (Alcaraz and García-Berthou 2007a; Alcaraz et al. 2008). The 2 captive experimental groups (from populations *A* and *B*) were bred and raised in captivity for a minimum of 5 years. They were housed in expansive water tanks characterized by minimal water flow, only enough for essential recirculation required for effective filtration and oxygenation. This system was meticulously designed to ensure and maintain a high standard of water quality. After sampling, both for wild-caught and captive-raised individuals, fish were promptly transported to the laboratory. Subsequently, they were given a 2-week acclimation period to adjust to the experimental conditions before the start of the experiment. Although the wild fish originated from a highly saline lagoon, their euryhaline nature (Moreno-Amich et al. 1999) facilitated a swift adaptation to the new laboratory conditions. Fish were held in 6 90 L glass aquariums under laboratory conditions and were separately distributed by population and sex (n = 15–17 fish per aquarium). Aquariums contained gravel substrate, conditioned water (conductivity ~ 320 µS cm^{-1}; pH ~7.6) and were supplied with recirculated, filtered freshwater (particle filtered and ozone sterilized), and vigorous aeration. Water changes of 30% of the total volume were conducted twice a week in each aquarium to assist with maintaining water quality. During the holding period, water temperature was set to 20 ± 1 °C. A natural photoperiod cycle (10–12 light: 12–14 dark) was used during the acclimation period.

From the second day of acclimation, fish were fed once a day with frozen bloodworms (*Chironomus* spp.) with a meal size of approximately 1.5–2% of their wet body mass. This amount of food represented a significant amount to maintain the fish body condition throughout the experiment, but it still was below from satiation levels. Fish were fasted 24 h prior to the respirometry trials, a post-feeding period that has been shown in several fish species to be long enough to avoid post-prandial effects (Secor 2009). No mortalities occurred during the acclimation period and all fish were visually in good health condition. Respirometry was conducted between autumn and early spring (from October to March) to avoid the breeding season of the species. At the end of the respirometry trials, all fish were kept in quarantine for at least one week, and then they were returned to the capture site.

After our previous studies on this species (Latorre et al. 2020; Rubio-Gracia et al. 2020), respirometry trials were conducted using one Blazka-type swim tunnel respirometer (Loligo® Systems, Viborg, Denmark). It consisted of a 170 mL tubular swimming chamber (100 mm length × 46.5 mm internal diameter) immersed in an external water bath containing 25 liter (L) of clean and aerated water. The external water bath was equipped with an automated Eheim pump that flushed constantly aerated and conditioned water (conductivity ~ 320 µS cm^{-1}; pH ~7.6) inside the swim tunnel respirometer at a rate of 5 L min^{-1}. Then, no water changes between trials were done. The external water bath was additionally connected to a plastic supply tank containing 300 L of air-saturated freshwater. An automated Eheim pump continuously provided freshwater from the supply tank to the external water bath. Then, the water was recirculated using a decantation system. In order to keep the temperature at 20 °C, which represents the middle range (10–32 °C) of the thermal niche of this species (Kottelat and Freyhof 2007), the supply tank was equipped with an automated liquid cooler (85 W, 972.46 BTU h^{-1}, J.P. Selecta®).

To generate the continuous laminar flow, a propeller was connected to the motor outside of the swim tunnel respirometer. Then, a rectilinear flow was made by placing a honeycomb plastic screen at the entrance of the swimming section. To determine the concentration of dissolved oxygen we used an optical fiber instrument (Witrox 1; Loligo® Systems, Tjele, Denmark), and to compensate the oxygen concentration regarding temperature and barometric pressure at real time we used an automated temperature probe (Pt1000 temperature sensor; Witrox 1; Loligo® Systems, Tjele, Denmark). Rates of oxygen consumption were measured using computerized, intermittent-flow respirometry. The swim tunnel respirometer was periodically flushed with aerated water

for 2 min (flush phase), followed by a 1 min closed mixing period and then 20 min of closed respirometry (measurement phase). We ensured that the oxygen concentration during the measurement phase was never below 7 mg L^{-1} to avoid any hypoxia-related effects. To calibrate oxygen consumption, we measured the highest water concentration value as 100% air-saturated and the lowest water concentration value as 0% using a solution of sodium sulfite (Na_2SO_3, 0.159 M) using the oxygen sensor.

At the beginning of each experiment, individuals were measured (standard body length, S_L) to the nearest 1 mm and then fish were placed individually into the swim tunnel respirometer. Firstly, fish was acclimatized for 2 h to an initial velocity of ca. 0.5 BL s^{-1} (body length, taken as the standard length of the fish, per second). After that, a critical swimming speed (U_{crit}) test was performed with step-wise increases in flow speed of approximately 1 BL s^{-1} every 20 min until the fish fatigued. Fatigue was defined as occurring when the fish could no longer actively swim against the current and was swept back against the mesh, usually after 4 or 5 h. The critical swimming speed (U_{crit}, cm s^{-1}) was calculated following (Brett 1964):

$$U_{\text{crit}} = U_{\text{f}} + U_{\text{i}} T_{\text{f}} / T_{\text{i}}$$

where U_f is the highest velocity maintained for a full 20-min period (cm s^{-1}), T_f is the time swum (min) at the last velocity increment, T_i is the interval time set (20 min in this case), and U_i is the velocity increment (BL s^{-1}). A few individuals that showed anomalous rheophilic behavior were omitted from the analyses (<3 fish per population and sex). Swimming speeds were not corrected for the "solid-blocking effect" because the cross-sectional area of the fish never overcame 10% of that of the swim tunnel respirometer (Bell and Terhune 1970). "Blank" observation was calculated for 10 min at the end of the each trial and corresponded to the background microbial respiration inside the swim tunnel respirometer. Estimates of microbial respiration ranged from 20% (high water flows) to 40% (low water flows) of the total oxygen consumption during the respirometry trials.

Oxygen consumption of the fish was calculated by fitting a linear regression of the oxygen concentration decline over time at each velocity. The resulting slope was used to calculate oxygen consumption rates (MO_2, in 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 swim tunnel respirometer (after subtracting the fish volume). For individual fish, MO_2 was used as a measure of metabolic rate (MR). Maximal metabolic rate (MMR) was defined as the highest MR during the swimming trial, which was usually close to the highest velocity (Srean et al. 2017; Rubio-Gracia et al. 2020). An exponential function was used to describe the relationship between MR and swimming speed (U) (Webb 1974; Tudorache et al. 2008):

$$\text{MR} = \text{SMR} \times e^{cU}$$

where SMR is the estimated standard metabolic rate at zero swimming speed, and c is the speed exponent. The absolute aerobic scope (AS) was calculated as the difference between MMR and SMR. Finally, at the end of the experiment individuals were weighed (wet mass) to the nearest 0.1 mg.

All details regarding the respirometry trials can be found in Supplementary Table S1 in the supporting information, as recommended by Killen et al. (2021).

Experiment 2: survival and body condition

After respirometry trials, a field experiment was conducted in the Bassa del Pi lagoon, also located in the Baix Empordà wetlands, NE Iberian Peninsula (42° 1′ 44.29″ N, 3° 11′ 19.57″ E). This lagoon is only separated 30 m from the Bassa del Fra Ramon lagoon, and they were both historically the same lagoon but were separated by a trail construction. As far as we know, no stocking of captive *A. iberus* has been carried out in the Bassa del Pi lagoon and both lagoons are eventually connected by floods of the salt marsh (mostly through sea storms). Therefore, we consider both lagoons as the same population (*B*). The water conductivity and temperature ranged from 10.0 mS cm^{-1} and 3 °C at the start of the experiment to 26.5 mS cm^{-1} and 17 °C at the end. This lagoon has no freshwater inlets, which explains its high salinity and *A. iberus* is the only fish species present.

We set up 10 floating mesocosms in the middle of the lagoon (Supplementary Figure S1). Mesocosms corresponded to a 6-sided cages were built out of 2-mm plastic mesh, which enclosed a volume of 0.6 m^3 (1 × 0.5 × 1.2 m). This mesh size prevented the entrance and escape of individuals, but allowed the passage of microinvertebrates, which constitute the main food of *A. iberus* (Alcaraz and García-Berthou 2007a). In each mesocosm we placed 10 females: in 5 of the mesocosms, wild females from local fish (hereafter, "population *B* wild"); and in the other 5 mesocosms, females from the same population *B* but raised in captivity in the Ichthyological Centre of Ebro Delta (as in the previous experiment; hereafter, "population *B* captive"). Note that the captive-bred individuals for this experiment were different from the ones used in the previous experiment (but from the same population *B*).

The experiment ran for over 4 months, from November 2017 to March 2018. Mesocosms were inspected weekly and once per month the survivors were counted, measured (S_L, in mm), weighed (in g) and returned to their mesocosm. The measurements were conducted without the use of anesthesia. At the end of the experiment, wild specimens were returned to the lagoon and the ones of captive origin were returned to the Ichthyological Centre of Ebro Delta.

Animal studies in captivity and fieldwork were approved by the Autonomous Government of Catalonia (ref. SF/1089), the University of Lleida's Animal Experimentation Commission (ref. CEA-OH/9673/1), and the Ebro Delta Natural Park as well as the Montgrí, les Illes Medes i el Baix Ter Natural Park (ref. 2017PNATMBTAUT0141).

Statistical analyses

In the first experiment, we used analyses of covariance (ANCOVA) to test for differences in swimming capacity and metabolic rates among population and sex groups (fixed effect factors), while accounting for fish mass (covariate). Critical swimming speed (U_{crit}), SMR, MMR, and AS were used as response variables. We used the full ANCOVA, which tests the interactions between the covariate (fish mass) and the categorical factors (population and sex), rather than the conventional ANCOVA design because the former model allows testing (rather than assuming) the homogeneity of slopes (García-Berthou and Moreno-Amich 1993) and because it always produced less (although similar) unexplained variation and significantly so for SMR (likelihood ratio test, $P = 0.027$). For

each source of variation, we calculated η^2 (eta squared) using package *heplots* (Fox et al. 2009) in R (R Core Team 2020). η^2 is a measure of effect size based on the sums of squares of ANCOVA that estimates the proportion of the total variance in the response variable associated with a certain predictor and that in the case of simple linear regression is identical to the coefficient of determination r^2. Because it has been suggested that variability could reflect acclimation abilities and should be examined in addition to central tendency (Devin et al. 2014), we also used Breusch–Pagan tests on the regression residuals of ANCOVAs and Fligner–Killeen tests for the factors (group and sex) to test for heteroscedasticity (both from the package *performance*); (Lüdecke et al. 2021).

In the field experiment, to test for significant differences in performance-related traits such as survival, length, mass, and body condition (mass adjusted for length) between wild and captive populations, generalized linear mixed models were used (Bates et al. 2014) with time (date) and group (wild vs. captive bred) as fixed factors and mesocosm as random factor (random slopes models). For body condition, length was added as a covariate. Binomial errors and function *glmer* from package *lme4* (Bates et al. 2014) were used for survival and function *lmer* for the other variables. We calculated *P* values for the mixed models with the *lmerTest* package (Kuznetsova et al. 2017). We also computed the marginal and conditional R^2 with the *MuMIn* package (Bartoń 2016). The marginal R^2 describes the variability explained only by the fixed effects, whereas the conditional R^2 describes the variability jointly explained by the fixed and the random effects.

All quantitative variables were $\log_{10}$-transformed to linearize the relationships and the assumptions of models were verified with residual plots from the package *performance*. All analyses and figures were obtained using R version 4.2.0 (R Core Team 2020). The raw data of both experiments are available at https://doi.org/10.6084/m9.figshare.23455955.v1.

Results

Experiment 1: respirometry trials and critical swimming speed

Respirometry and critical swimming speed trials showed that mass was always important and significant for these variables except for absolute aerobic scope (AS) (Table 1). Notably, standard metabolic rate (SMR) and maximum metabolic rate (MMR) were less dependent on mass than critical swimming speed (U_{crit}). Captive individuals exhibited lower values of AS in comparison to their wild counterparts, with no significant effects of sex (Figure 1D, Tables 1 and 2). U_{crit} did not display clear effects of sex either but was highest for captive individuals of population *A*, intermediate for wild individuals of population *A*, and lowest for captive individuals of population *B* (Figure 1A, Tables 1 and 2). By contrast, the outcomes for SMR and MMR were influenced by sex, with significant group × sex interaction (Table 1) because wild individuals and females generally had higher values but captive-bred males of population *A* had higher mass-adjusted rates than females (Figure 1, Table 2). These differences are considerable given the log scales (Figure 1) and captive-bred individuals have 30–76% lower SMR and MMR on average (Table 2).

Specifically, a likelihood ratio test showed that the general ANCOVA model explained more variation than the standard design (with no interactions) for standard metabolic rate (SMR) ($P = 0.027$) and the mass × population interaction was significant for this variable (Table 1), indicating that the slopes of the 3 experimental groups were different (Figure 1B). By contrast, there were no clear differences in slopes for the other 3 variables (Table 1). The overall explained variation was lowest for U_{crit} and highest for maximum metabolic rate (MMR) (Table 1). The experimental group was the most important source of variation for all variables except for critical swimming speed (U_{crit}) (see η^2 in Table 1). Sex was less important and only showed clear population × sex interaction for SMR and MMR.

There was no evidence of heterocedasticity for the regression residuals of these 4 ANCOVA models (Breusch–Pagan tests, *P* values = 0.07-0.95), suggesting that differences in variability were negligible after accounting for central tendency (Figure 1). By contrast, the variances of SMR (but not the 3 other variables) clearly varied with group and sex (Fligner–Killeen test, $P = 0.036$), because they tended to be smaller for captive fish (Supplementary Figure S2, Table 2). For instance, the coefficient of variation of SMR was 51 and 49% for the captive males of the 2 populations versus 81% for the wild

Table 1. Analyses of covariance of swimming performance and the 3 metabolic traits of Spanish toothcarp with the experimental group (population *A* captive, population *A* wild, and population *B* captive) and sex as factors and fish mass as covariate. The explained variation (adjusted R^2) is given in parentheses. η^2 (eta squared) is the proportion of the total variance in the response variable that is associated with a certain source of variation. Response variables and covariate were $\log_{10}$-transformed for the analyses

Source of variation	U_{crit} (0.289)			MMR (0.376)			SMR (0.458)			AS (0.328)		
	η^2	d.f.	*P*	$\eta2$	d.f.	*P*	$\eta2$	d.f.	*P*	$\eta2$	d.f.	*P*
Mass (*M*)	0.19	1	***	0.06	1	***	0.10	1	***	0.01	1	0.25
Group	0.15	2	***	0.45	2	***	0.21	2	***	0.35	2	***
Sex	0.01	1	0.25	0.01	1	0.31	0.02	1	0.07	0.00	1	0.68
M × group	0.02	2	0.18	0.00	2	0.80	0.06	2	**0.01**	0.03	2	0.14
M × sex	0.00	1	0.38	0.00	1	0.57	0.00	1	0.39	0.00	1	0.40
Group × sex	0.01	2	0.56	0.03	2	**0.03**	0.12	2	***	0.00	2	0.89
M × group × sex	0.01	2	0.60	0.00	2	0.75	0.02	2	0.20	0.00	2	0.78
Residuals		109			97						93	

Abbreviations are: U_{crit} (critical swimming speed), SMR (standard metabolic rate), MMR (maximal metabolic rate), and AS (absolute aerobic scope).
*** indicates $P < 0.001$; significant *P* values ($P < 0.05$) are bolded

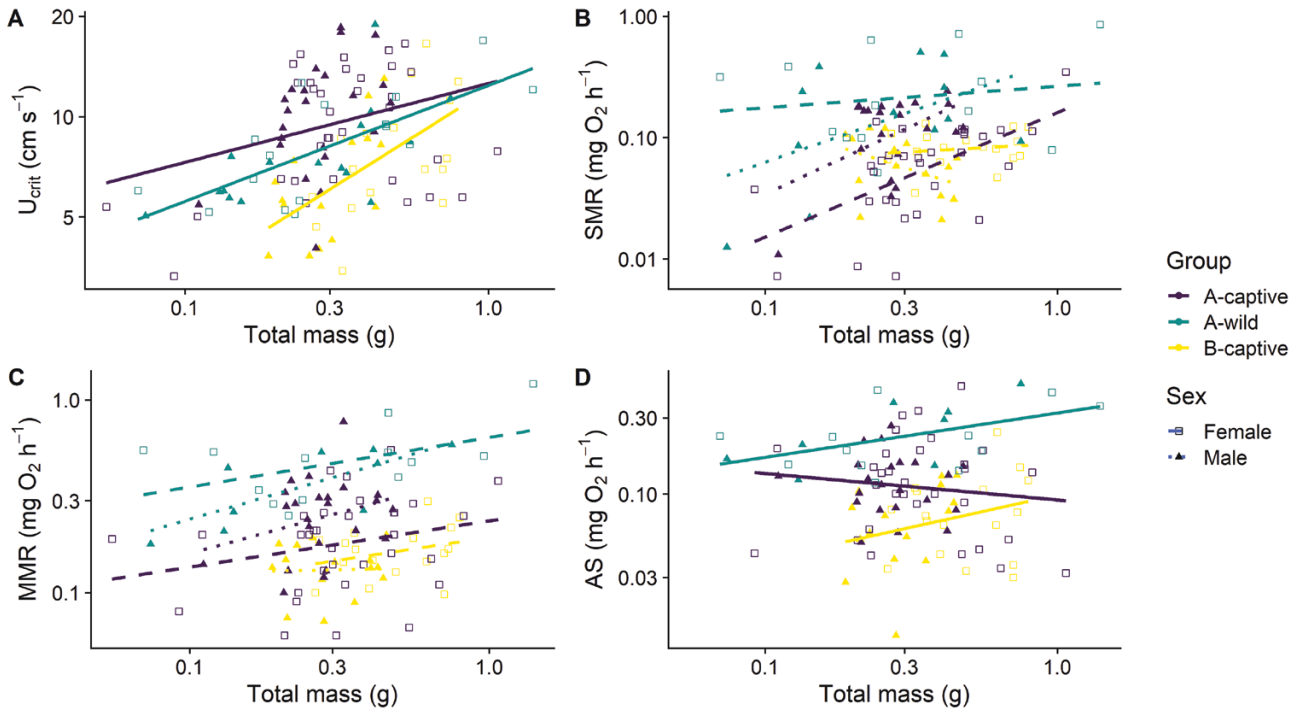

Figure 1. Relationship of swimming performance and the 3 metabolic traits of Spanish toothcarp with fish mass by experimental group (population A captive, population A wild, and population B captive) and sex. See Table 1 for abbreviations of response variables. The regression lines by group are shown depending on the statistical results of Table 1. Note the log scales of all axes. Population A corresponds to Alt Empordà wetlands (Clot de la Llúdriga lagoon) whereas population B corresponds to Baix Empordà wetlands.

males, whereas the equivalent figures for females were 31, 86, and 87%.

Experiment 2: Survival and body condition

There were no clear differences in survival of the wild and captive individuals or along the field experiment because most fish survived (Table 3), although 2 of the captive-fish mesocosms showed decreases in the number of individuals (Figure 2). The marginal R^2 was very similar to the conditional R^2 (Table 3), suggesting negligible differences among mesoscosms.

The mean mass of individuals clearly increased along the experiment with significant time × group interaction (Table 3), because captive individuals, which were smaller at the start, grew much more (mean = 0.30 g) than wild individuals but were still smaller at the end of the experiment (Figure 3A). The differences among mesocosms within treatments (random effects) were small and not significant, in agreement with the small differences between marginal and conditional R^2 (Table 3). The results for mean length were similar, with increases along the experiment (Table 3), particularly for the captive-bred population (Figure 3A). Body condition (mass adjusted for length) showed significant time × population × length interaction (Table 4), i.e., heterogeneous slopes that varied with time because at the beginning of the experiment, large captive-bred individuals showed lower condition than wild individuals of the same length, with negligible differences for small fish (Figure 4). Along the experiment, these differences vanished, and no body condition differences were observed between wild and captive-bred individuals at the end of the experiment (Figure 4). Overall, these results suggest that captive-bred individuals grew more with time but more in mass than in length.

Discussion

Captive breeding and subsequent stocking has been generally used to enhance or restore wild populations (Philippart 1995; Fraser 2008). As in Latorre et al. (2020), we demonstrated that individuals of this endangered species (*A. iberus*) raised in captivity during almost 5 years, showed lower metabolic rates and aerobic scope than wild individuals, and less variability in SMR. These changes could compromise the fitness and viability of the captive populations when they are reintroduced into the wild (Kohane and Parsons 1988; Frankham and Loebel 1992; Ruzzante and Doyle 1993; Lynch and O'Hely 2001). It seems that captivity conditions likely reduce individual metabolism (Du Preez 1987; Wieser et al. 1992; Biro and Stamps 2010; Auer et al. 2015). Previously, Latorre et al. (2020) suggested that the lower metabolic rates of the captive individuals of population *A* (Clot de la Llúdriga) of the studies species at the University of Girona might be related to the low food availability for those individuals, compared with the wild ones. However, in this study we also analyzed captive individuals from the Ichthyological Centre of Ebro Delta with no food restriction that also showed lower metabolic rates than wild specimens. Alternatively, such differences in metabolic rates between wild and captive individuals could be due to differences in food availability, being more variable in the wild than in captivity, and that food quality or nutrient availability could be different between environments. In addition, differences in metabolic rates and aerobic scope between wild and captive-bred individuals might be due to the influence of environmental conditions on development programming and epigenetic factors, possibly causing metabolic changes later in life or in the next generations (Cavalieri and Spinelli 2017; Berbel-Filho et al. 2020). Aerobic scope reflects the energy that an individual can invest into somatic growth and gamete

Table 2. Critical swimming speed and metabolic traits of Spanish toothcarp for the 3 experimental groups (population *A* wild, population *A* captive, and population *B* captive), separately for females and males

Variable	*A* wild females (*n* = 16)	*A* captive females (*n* = 34)	*B* captive females (*n* = 17)	*A* wild males (*n* = 16)	*A* captive males (*n* = 23)	*B* captive males (*n* = 15)
S_L (cm)						
Mean (SE)	2.36 (0.14)	2.46 (0.07)	2.77 (0.09)	2.06 (0.09)	2.30 (0.05)	2.37 (0.07)
Range	1.6–3.6	1.4–3.5	2.0–3.2	1.6–3.2	1.7–2.7	1.9–2.7
T_L (cm)						
Mean (SE)	2.75 (0.17)	2.89 (0.12)	3.32 (0.12)	2.51 (0.12)	2.84 (0.06)	2.86 (0.09)
Range	2.0–4.4	0.170–4.1	2.5–4.0	2.0–4.0	2.2–3.4	2.3–3.4
Mass (g)						
Mean (SE)	0.391 (0.087)	0.380 (0.036)	0.510 (0.045)	0.302 (0.046)	0.287 (0.019)	0.309 (0.025)
Range	0.070–1.400	0.055–1.070	0.262–0.795	0.074–0.750	0.111–0.476	0.188–0.453
U_{crit} (cm s^{-1})						
Mean (SE)	8.809 (0.838)	9.927 (0.648)	8.225 (0.850)	8.006 (0.856)	10.668 (0.806)	6.740 (0.718)
Range	5.100–16.950	3.320–16.590	3.450–16.575	5.040–18.900	4.030–18.480	3.820–12.994
SMR (mg O_2 h^{-1})						
Mean (SE)	0.307 (0.074)	0.074 (0.011)	0.085 (0.006)	0.209 (0.049)	0.130 (0.014)	0.066 (0.009)
Range	0.051–0.856	0.007–0.348	0.035–0.131	0.012–0.506	0.011–0.241	0.021–0.120
MMR (mg O_2 h^{-1})						
Mean (SE)	0.520 (0.072)	0.208 (0.020)	0.170 (0.013)	0.388 (0.053)	0.272 (0.029)	0.137 (0.010)
Range	0.251–1.212	0.060–0.550	0.098–0.298	0.178–0.583	0.100–0.770	0.071–0.199
AS (mg O_2 h^{-1})						
Mean (SE)	0.239 (0.033)	0.139 (0.018)	0.084 (0.013)	0.266 (0.045)	0.125 (0.012)	0.072 (0.009)
Range	0.118–0.447	0.032–0.474	0.030–0.244	0.122–0.490	0.050–0.266	0.013–0.131

Means, standard errors (SE), and ranges (minimum and maximum) are shown. S_L is standard length and T_L is total length. See Table 1 for other abbreviations. Population *A* corresponds to Alt Empordà wetlands (Clot de la Llúdriga lagoon), whereas population *B* corresponds to Baix Empordà wetlands.

Table 3. Generalized linear mixed models of fish survival, mass, and length of Spanish toothcarp in the field experiment at Baix Empordà wetlands (population *B*) from November 2017 to March 2018 using time and experimental group (captive-bred vs. wild) as categorical factors

Source of variation	Survival (0.630, 0.648)			Mass (0.342, 0.347)			Length (0.368, 0.420)		
	$\chi 2$	d.f.	*P*	*F*	d.f.	*P*	*F*	d.f.	*P*
Time	0.304	3	0.959	13.3	3, 101	**<0.001**	15.43	3, 71	**<0.001**
Group	0.000	1	1.000	107.1	1, 9	**<0.001**	39.78	1, 7	**<0.001**
Time × group	0.000	3	1.000	3.3	3, 101	**0.025**	2.72	3, 71	0.051

Mesocosm was used as random factor (random-slope model). The 2 values in parentheses are the marginal and conditional R^2, respectively. Significant *P* values ($P < 0.05$) are bolded

production, and other fitness related functions (Paschke et al. 2018). Thus, we could assume that captive-bred fish might have greater costs, as these fish will have less energy available for somatic growth and reproduction. However, differences in metabolic rates between wild and captive populations could also be explained by the low energy requirements of captive individuals. This could be attributed to the highly favorable environmental conditions in captivity such as the absence of predators and competitors, as well as the presence of more stable abiotic conditions that prevent individuals from constantly adjusting their metabolic rates. In our study sites, predation in the wild is mostly due to aquatic birds and eventually eels (scarce in the lagoons), but was probably artificially low in our mesocosms as in both captive conditions. Changes in metabolic traits have been suggested to affect individual behavior and performance/fitness-related traits such as survival, growth, and body condition (Biro and Stamps 2010; Burton et al. 2011), which may compromise the success of the reintroductions.

In contrast to Rubio-Gracia et al. (2020), we observed clear differences in SMR and MMR between sexes for the studied species. These differences also depended on raising conditions (captivity vs. wild). The disparities in metabolic rates between sexes have been proposed to be linked to various energy investments, particularly in reproduction and associated with gamete production (Hayward and Gillooly 2011). However, the precise factors contributing to the observed pattern remain unclear. Conducting specific analyses on reproductive investment at the time of measurement could provide valuable insights into elucidating the diverse

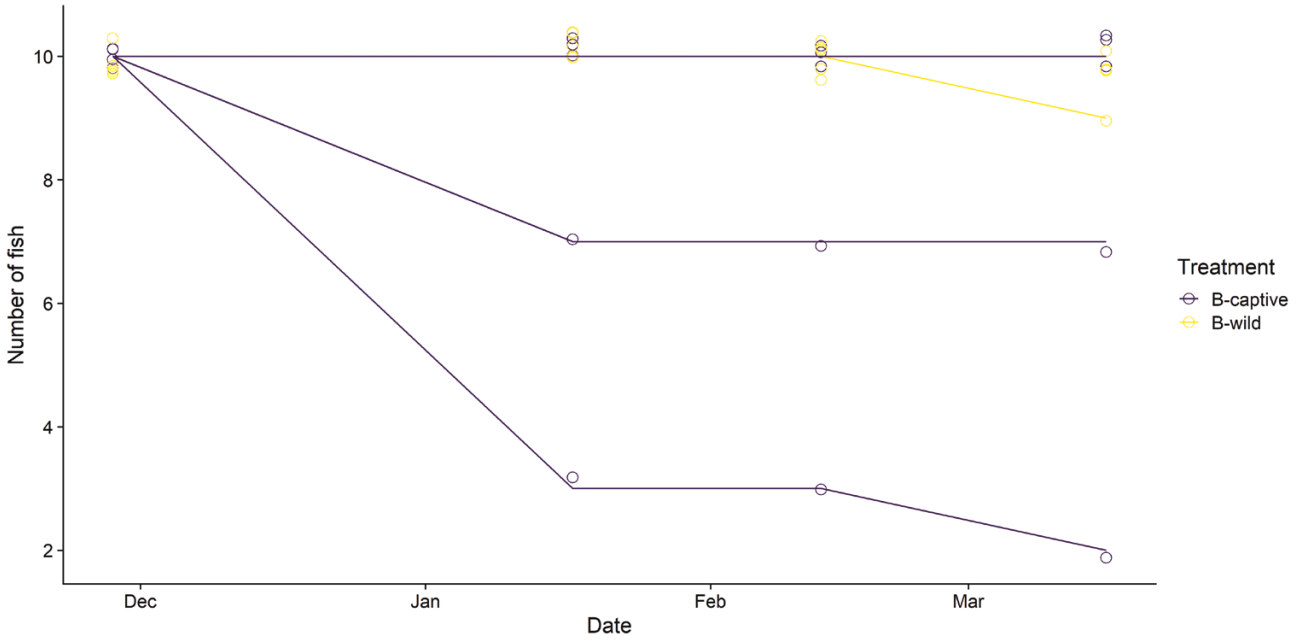

Figure 2. Survival of captive-bred versus wild individuals of Spanish toothcarp from population B in the field experiment at Bassa del Pi lagoon from November 2017 to March 2018. Note that all fish survived in 7 out of 10 mesocosms and that the circles are jittered to facilitate distinction.

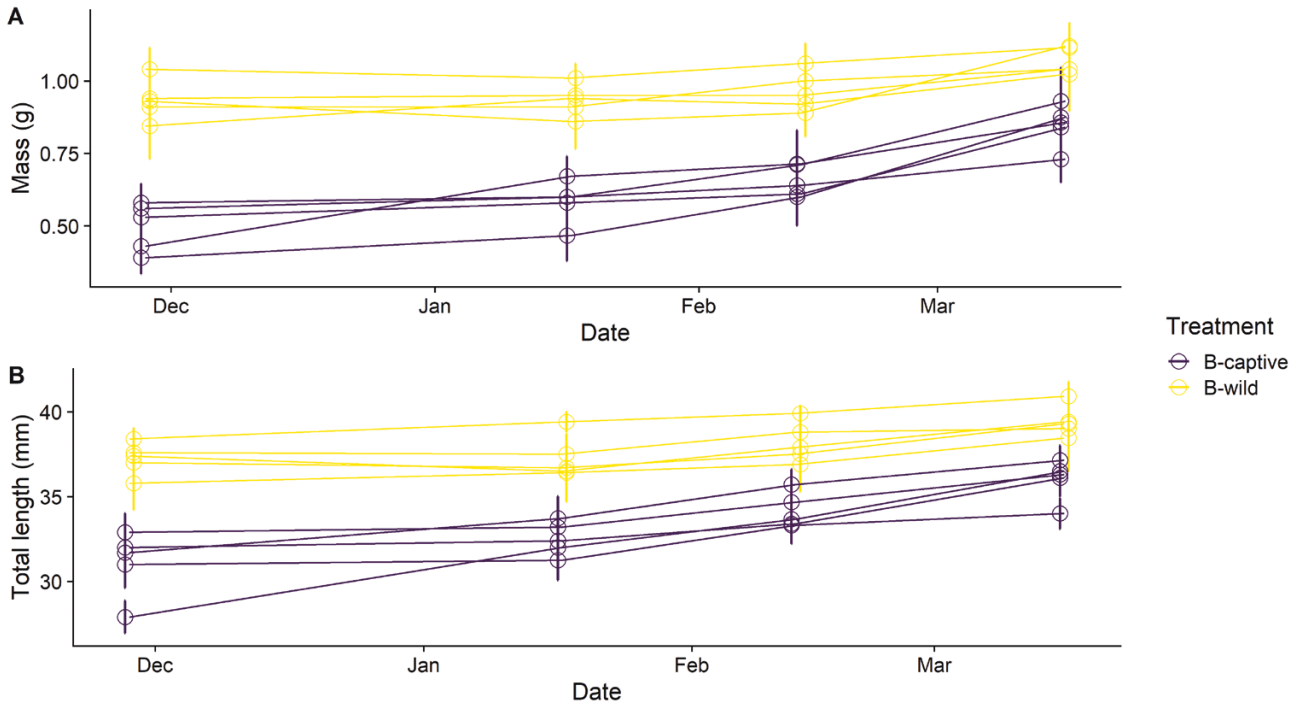

Figure 3. Growth in mass and length of captive-bred versus wild individuals of Spanish toothcarp from population B in the field experiment at Bassa del Pi lagoon from November 2017 to March 2018. Means and standard errors are shown for each mesocosm.

possibilities underlying this discrepancy in metabolic rates between sexes.

Swimming performance (i.e., U_{crit}) was low for all groups in comparison with more rheophilic species, in agreement with the slow-flowing habitat preferences of this species (Alcaraz et al. 2008; Latorre et al. 2020; Rubio-Gracia et al. 2020). Moreover, differences in swimming capacity between groups were more related to geographical origin differences than to rearing conditions (wild vs. captive). Wild and captive individuals from population *A* (Alt Empordà wetlands) had higher swimming capacity than captive individuals from population *B* (Baix Empordà wetlands). Rubio-Gracia et al. (2020) found that in this species 67% of the aerobic metabolism is used for swimming, being much higher compared with other species such as *Gambusia holbrooki*. Accordingly, the lower swimming capacity of captive individuals from population *B* at the Ichthyological Centre of Ebro Delta might be consequence of the reduced metabolism of this population

compared with the wild and captive individuals of the other population. This might not suppose a limitation for captive individuals when they are released into the wild, because *A. iberus* usually inhabit slow-flowing habitats with high vegetation density. In this kind of habitats, it might be more advantageous for this species to have greater maneuverability and burst-swimming to escape from predators (Reidy et al. 2000) instead of higher swimming resistance (Alcaraz and García-Berthou 2007b; Rubio-Gracia et al. 2020).

We found no clear differences in survival between wild and captive individuals once they were reintroduced into the wild. Throughout the experiment, individuals raised in captivity were significantly smaller but grew more in mass than wild ones. Similarly, captive-bred individuals increased more in mean length than wild individuals. Regarding body condition, no clear differences were observed at the end of the experiment between wild and captive-bred individuals.

Table 4. Linear mixed model of fish mass of Spanish toothcarp in the field experiment at Baix Empordà wetlands (population *B*) from November 2017 to March 2018 using time and experimental group (captive-bred vs. wild) as categorical factors and length as covariate

Source of variation	*F*	d.f.	d.f.	*P*
Time	3.53	3	310.8	**0.015**
Group	4.17	1	334.1	**0.042**
Length	1094.59	1	334.7	***
Time × group	3.24	3	310.8	**0.023**
Time × length	3.63	3	310.7	**0.013**
Group × length	4.50	1	334.7	**0.035**
Time × group × length	3.45	3	310.7	**0.017**

Mass and length were $\log_{10}$-transformed for the analysis. Mesocosm was used as random factor (random-slope model). Marginal $R^2 = 0.846$; conditional $R^2 = 0.857$. *** indicates $P < 0.001$; significant P values ($P < 0.05$) are bolded.

Thus, captive individuals showed higher performance traits (i.e., growth in mass and length) under natural conditions than wild individuals, which might be related to the differences found in metabolic traits between wild and captive-bred populations. It has been suggested that fitness consequences of a given metabolic phenotype may be context-dependent (Burton et al. 2011; Auer et al. 2015). In favorable environmental conditions, individuals with higher metabolic rates can optimize their fitness by effectively offsetting the associated elevated maintenance costs (Burton et al. 2011). On the other hand, in poor environmental conditions, individuals with lower metabolic rates enjoy a fitness advantage due to their reduced maintenance requirements (Burton et al. 2011). The advantageous lower energy requirements of captive individuals, resulting from reduced metabolic rates, become particularly beneficial in mesocosm conditions, that were more challenging than in captivity. This phenomenon may clarify the superior performance observed in the captive-bred fish within the mesocosm environment. In contrast, higher metabolic rates of wild individuals might be disadvantageous under mesocosms conditions, which might explain the lower performance observed in the wild population. These findings agree with those suggesting that the fitness consequences of a given metabolic phenotype might depend on the environmental conditions to that individuals are exposed (Burton et al. 2011; Auer et al. 2015). Studies using mesocosms provide reliable information related to the conditions that individuals are exposed to (i.e., abiotic conditions) and help us understand the consequences of having high or low metabolic rates under certain environmental conditions.

High survival and high growth in mass and length might enhance the performance/fitness and success of captive individuals when they are released into the wild. These results contrast with previous studies that suggested that rearing fish in captivity could negatively affect the population's fitness (i.e., survival, growth, or reproduction) when they are released into the wild (Reisenbichler and Rubin 1999; Berejikian &

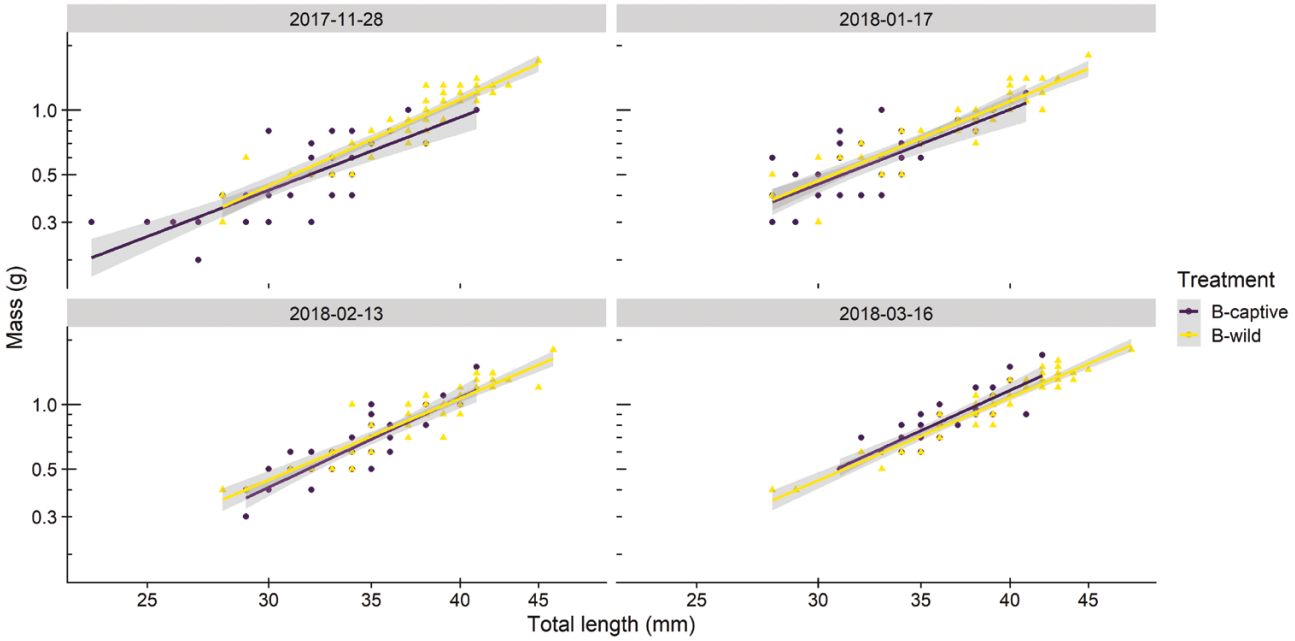

Figure 4. Mass–length relationships of captive-bred versus wild individuals of Spanish toothcarp from population B in the field experiment at Bassa del Pi lagoon from November 2017 to March 2018. The regression lines by group are shown. Note the log scales of all axes.

Ford 2004; Araki et al. 2008; Gavery et al. 2018). It is noteworthy that captive-bred fish were smaller at the beginning of the experiment, which might have contributed to their higher growth rates. Another possible explanation for this pattern is that the wild (larger) fish might have reached maturity earlier and thus lowering the growth rate. Furthermore, compensatory growth might also be an alternative mechanism behind the increase in body mass in relation to body length, where body mass typically increases rapidly prior to the investment in structural growth (i.e., length). However, the success of captive populations to the wild could be threatened if reintroductions do not occur under adequate environmental conditions (Burton et al. 2011; Auer et al. 2015). Natural conditions might be more challenging, exhibiting greater variability, compared with the controlled environment of captivity, therefore the lower energy requirements of captive individuals could result in higher performance/fitness. Consequently, it would be of great importance to select the best period to release captive individuals into the wild. If captive individuals exhibit lower metabolic rates than wild ones, reintroductions should take place under less favorable environmental conditions (i.e., autumn or winter) to enhance performance/fitness-related traits and consequently, reintroduction success. On the other hand, under more favorable environmental conditions (spring), wild individuals exhibiting higher metabolic rates would be better competitors and would reproduce better (Burton et al. 2011), indirectly affecting the viability of the captive population and reintroduction.

Throughout the entire duration of the mesocosm experiment, individuals bred in captivity consistently exhibited smaller sizes compared with their wild counterparts. It has been suggested that individual size at release might affect intra- and inter-specific interactions (Cutts et al. 1999; Johnsson et al. 1999; Einum and Fleming 2001). Magellan and García-Berthou (2015) suggested that smaller individuals of *A. iberus* might be more vulnerable in the presence of the invasive species *Gambusia holbrooki*, which negatively affects survival rates. Mesocosms did not allow captive individuals to interact with wild individuals or with other fish species. Nevertheless, in natural conditions, restocking with larger individuals or within the size range of wild individuals is likely to result in increased survival for reintroduced and native populations (Einum and Fleming 2001; Magellan and García-Berthou 2015).

In summary, captive breeding conditions caused physiological changes in *A. iberus* (i.e., mass, length, and body condition), which might lead to performance/fitness differences when wild and captive populations were maintained under the same semi-controlled environmental conditions (mesocosms). Under experimental conditions, the captive-bred individuals showed lower metabolic rates, which might enhance fitness-related traits when individuals are exposed to less favorable conditions in the wild. Thus, although rearing *A. iberus* in captivity seemed not to affect their success when they were released into the wild, reintroduction success may depend on the environmental conditions at the time of release. Studies using mesocosms allow us to control many environmental factors (e.g., inter-specific competition, predation), thus permitting us to understand how changes in metabolic traits in captive conditions might affect performance/fitness in the natural environment. Furthermore, to ensure the conservation and recovery of endangered species it is necessary to evaluate the effectiveness of reintroduction programs and thus to understand how captive-bred individuals respond to intra- or inter-specific interactions and challenging abiotic conditions.

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Conflict of Interest statement

The authors declare no conflict of interest.

Authors' Contributions

G.M., D.L., and R.M. did the field and laboratory work. E.G.B. made the statistical analyses, prepared the illustrations, and wrote the manuscript. A.V.G. conceived the idea of the study, and G.M. and A.V.G. wrote the first draft of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Ethics Statement

Animal research under captive conditions and fieldwork were authorized by the Autonomous Government of Catalonia (ref. SF/1089), the Commission of Animal Experimentation of the University of Lleida (ref. CEA-OH/9673/1), the Ebro Delta Natural Park and the Montgrí, les Illes Medes i el Baix Ter Natural Park (ref. 2017PNATMBTAUT0141).

Data Availability

All the raw data used for statistical analyses is available at figshare (https://doi.org/10.6084/m9.figshare.23455955.v1).

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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