UNIVERSIDADE DE ÉVORA

ESCOLA DE CIÊNCIAS E TECNOLOGIA

DEPARTAMENTO DE BIOLOGIA

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INSTITUTO SUPERIOR DE AGRONOMIA

Effects of flow regulation in the life-cycles of a mediterranean cyprinid species, the Iberian chub (*Squalius carolitertii* Doadrio, 1987)

Carlos Manuel Engeitado Alexandre

Orientação: Professor Doutor Pedro Raposo de Almeida

Mestrado em Gestão e Conservação de Recursos Naturais

Dissertação

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EFFECTS OF FLOW REGULATION IN THE LIFE-CYCLES OF A MEDITERRANEAN CYPRINID SPECIES, THE IBERIAN CHUB (*SQUALIUS CAROLITERTII* DOADRIO, 1987)

ABSTRACT

Streamflow is considered a driver of inter and intra-specific life-history differences among freshwater fish. Therefore, dams and related flow regulation, can have deleterious impacts on their life-cycles. The main objective of this study is to assess the effects of flow regulation on the growth and reproduction of a non-migratory fish species. During one year, samples were collected from two populations of Iberian chub, inhabiting rivers with non-regulated and regulated flow regimes. Flow regulation for water derivation promoted changes in chub's condition, duration of gonad maturation and spawning, fecundity and oocyte size. However, this non-migratory species was less responsive to streamflow regulation than a migratory species analysed. Findings from this study are important to understand changes imposed by regulated rivers on fish and can be used as guidelines for flow requirements implementations.

KEY-WORDS: Life-history, dams, streamflow regulation, Northern Iberian chub, Iberian rivers.

EFEITOS DA REGULARIZAÇÃO DE CAUDAL NOS CICLOS DE VIDA DE UMA ESPÉCIE CIPRINÍCOLA DA REGIÃO MEDITERRÂNICA, O ESCALO DO NORTE (*SQUALIUS CAROLITERTII* DOADRIO, 1987)

RESUMO

O caudal é um dos fatores responsáveis pelo funcionamento dos ciclos de vida das espécies piscícolas dulciaquícolas. As barragens, e a regularização de caudal associada, podem ter impactes nos ciclos de vida destas espécies. O objetivo deste estudo prende-se com a avaliação dos efeitos da regularização de caudal no crescimento e reprodução de uma espécie piscícola não-migradora. A análise de amostras recolhidas em populações de escalo do Norte provenientes de dois rios de caudal regularizado e não regularizado, identificaram impactes significativos a nível da condição corporal, da maturação das gónadas e desova, da fecundidade e da dimensão dos oócitos. Esta espécie não-migradora parece ser menos responsiva à artificialização do caudal que uma espécie migradora previamente analisada. Estes resultados permitem compreender as alterações impostas pela regularização do caudal e podem ser usados em programas de reabilitação fluvial.

PALAVRAS-CHAVE: Estratégias de vida, barragens, regularização de caudal, escalo do Norte, rios Ibéricos.

1. INTRODUCTION

Streamflow patterns have a major influence on shaping the life-history strategies of aquatic species (Poff *et al*., 1997). Although is well stablished that temperature regimes influence the life-cycle patterns of many stream and river animals (Olden & Naiman, 2010), the influence of water level fluctuations and flow disturbances, as well as its frequency, intensity and timing, is also important for the life-cycle development of the majority of existing groups of freshwater biota (Resh *et al*., 1988). Seasonal timing and predictability of the natural flow regime are particularly critical because the life-cycles of many aquatic species are timed to avoid or take advantage of particular flow conditions (Poff *et al*., 1997). For freshwater fishes in particular, flow plays an important role in the lives of fish with critical life events linked to flow regime (e.g., reproduction, spawning behavior, larval survival, growth patterns and recruitment) (Junk *et al*., 1989; Humphries *et al*., 1999). Many of these life events are synchronized with temperature and day-length such that changes in flow regime that are not in natural harmony with these seasonal cycles may have a negative impact on aquatic biota (Bunn & Arthington, 2002).

Streamflow, temperature and habitat alterations resulting from dam construction and regulation can take many different forms, usually according to the type of river where these infrastructures are built or their operation mode and purpose (Bunn & Arthington, 2002). In short, some type of dams tends to homogenise and stabilize river flow (i.e. storage, flood control or derivation dams), others may cause an inversion of the natural streamflow pattern, with higher flows in summer and lower flows in winter (i.e. irrigation dams) and others, like hydroeletric facilities, affect the natural rate of environmental change, usually causing extreme and umpredictable daily variations in water level, temperature and available habitat (for a review of the effects of several dams please check e.g., Poff & Zimmerman, 2010; Arthington, 2012; Alexandre *et al*., 2014a). Thus, considering the link between fish life-cyles and flow or temperature patterns, dam operations that significantly artificialize riverine flow and thermal regimes can result in effective and persistent effects on fish faunas at local and regional scales (Olden & Naiman, 2010). For example, in cases where fish species use seasonal peak flows as a cue for egg hatching, migration or spawning, river regulation that eliminates or reduces these peaks can directly reduce local populations of such species (Naesje *et al*., 1995; Welcomme *et al*., 2006). Sudden flooding or more gradual rising river flows, may act as the spawning trigger for fishes in large floodplain rivers with a predictable annual flood (Junk et al., 1989; Welcomme et al., 2006).

Furthermore, effects of flow regulation in the life-cycles of freshwater fishes may also come in an indirect way, since entire food webs, not just single species, may be modified by altered flow timing. In some regulated rivers, characterized by an inversion of the natural streamflow pattern, the shift of most scouring flows from winter to summer may indirectly reduce the growth rate of riverine fish species by increasing the relative abundance of predator-resistant invertebrates, diverting energy away from the food chain that, ultimately, leads to fish (Wootton et al., 1996). In non-regulated rivers, high winter flows reduce these predator-resistant insects and favor species that are more palatable to fish (Bunn & Arthington, 2002).

Life-history characteristics of riverine fish are well studied and well-suited as a platform to test general relationships between flow regime and biological communities (e.g., Winemiller & Rose, 1992; Lamouroux *et al*., 2002; Mims & Olden, 2012; 2013; Alexandre *et al*., 2015a). However, most models and other studies addressing this relationship were developed to analyse variability in life-cycle traits at the assemblage level and the aspects of the links between fish bio-ecological cycles and environment remain relatively unknown at the population level. Intra-specific variability in the life-histories of fish determined by environmental conditions has been demostrated to occur in numerous cases (e.g., Spranza & Stanley, 2000; Blanck *et al*., 2007; Alexandre *et al*., 2015a; 2015b). Described patterns are, generally, in accordance to previous theories about fish life-history strategies (Winemiller & Rose, 1992), and include changes in diet composition and feeding strategy (Alexandre *et al*., 2015b); differences in age at first reproduction due to flow or thermal effects or food availability (Alexandre *et al*., 2015a), longevity (Baltz and Moyle, 1984), brood size and size at first reproduction (Baylis et al., 1993).

The majority of the existing studies linking natural and artificial streamflow and temperature variability, caused respectively by regional differences or dam construction and operation, amd fish life-history strategies have been conducted only in North American basins, mostly for large migratory species (especially salmonids) or across small areas with different habitat types (e.g., Mims & Olden, 2012; 2013), and there is scarce number of studies dealing with intra-specific and population-based differences in fish, especially mediterranean cyprinids, life-histories across large-scale gradients of streamflow variability, especially the bio-ecologic deviations related with the effect of human-altered streamflows (e.g., Weisberg and Burton, 1993; Torralva et al., 1997). Recently, a study by Alexandre *et al*. (2015a), developed in several non-regulated and regulated mediterranean watercourses, identified significant differences in growth and reproduction patterns, as well as somatic condition and longevity, for a cyprinid fish species from the Iberian Peninsula, for which the authors gave credit to the streamflow and temperature regulation operated by two different types of dams. This study however, was focused on a typical large potamodromous species, the Iberian barbel (*Luciobarbus bocagei* Steindachner, 1864), which are usually more plastic, in phenotypic terms, and responsive to environmental variability, and it remains to be evaluated if a similar level of response also happens in smaller, more resident, species, which are not so dependent of these flow and thermal cues to complete their life-cycles.

The main objective of this study is to assess the influence of hydrological variability, caused by flow regulation, in the life-history characteristics of a typical mediterranean resident fish species, the Northern Iberian chub (*Squalius carolitertii* Doadrio, 1987), a cyprinid species endemic to the northwestern part of the Iberian Peninsula. More specifically, this study aims to: i) assess if and how the flow regulation caused by a dam operating for water storage and derivation purposes, affects age, growth and reproduction traits of this non-migratory species; and ii) evaluate the relationship between the biological cycles of the target species and several flow and temperature components.

Altough being usually less studied than large migratory species, resident fish species, like the Iberian chub, have drawn the attention of conservation agencies and management authorities because they usually exhibit a high rate of decline linked to environmental perturbation (Maia *et al*., 2006). Therefore, it is essential to study their responses to environmental disturbance, especially the one caused by antropogenic actions, such as dam construction and operation, whose role and effects on aquatic ecossytems can be mitigated through suitable management actions.

2. MATERIAL AND METHODS

2.1. STUDY AREA

This study was conducted in two river systems (Figure 1), one regulated and other non-regulated, located in the northwestern part of the Iberian Peninsula (IP), an area with strong Atlantic influence where rivers have a permanent flow throughout the year, which contrasts with the rest of the country where rivers tend to follow the traditional mediterranean pattern of drying during summer periods. River systems were selected following a criterion of minimum evidence of human disturbance (flow regulation aside) such as physical habitat modifications, point-sources of pollution or agricultural run-off.

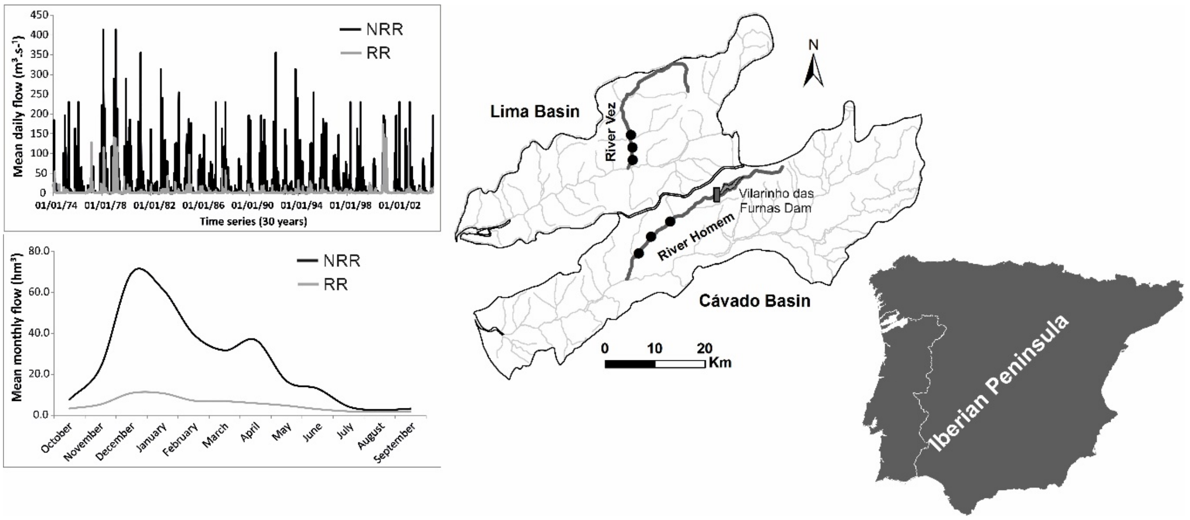

Figure 1. Location of the study area in both river systems, sampling sites (●) in the selected regulated and nonregulated rivers and respective hydrograms of mean daily discharge ($m^3.s^{-1}$) and monthly flow volume (hm^3). In the charts presented, ▬ and ▬ represent, respectively, the nonregulated (NRR) and regulated (RR) watercourses of each system.

The "treatment river" selected to asses the effects of flow regulation on chub's life-cycle was River Homem (Regulated River, RR), located in the Cávado river basin, which has approximately 49 km of extension and a drainage area of 257 km^2 (SNIRH, 2013). Since 1972, its flow is being regulated by Vilarinho das Furnas dam, which is operated mainly for water storage and derivation for its larger and more productive counterpart, Caniçada Dam. Vilarinho das Furnas releases a constant hypolimnetic flow, set in order to fulfil minimum ecological flow requirements (Environmental Flow Regime; EFR), which reduces and homogenises the river flow, while severely diminishing the variability of habitual and extreme values throughout the year. To act as “reference” in this study we selected River Vez (Non-regulated River, NRR), a non-regulated river located in River Lima basin, with 38 km of length and a drainage area of 264 km^2. Despite being located in disctinct river basins (i.e. Cávado basin did not had any non-regulated similar watercourse) both studied rivers are similar in terms of abiotic and biotic (i.e. fish assemblage) tipologies (INAG, 2008; Matono *et al.,* 2009; Alexandre *et al.*, 2013), which allows to compare them, with a high level of confidence, for the purposes of this study.

A more detailed characterization of the studied rivers and of the main streamflow and thermal regimes differences between them is presented by Alexandre *et al*. (2013; 2015a; b). In short, only slight differences are observed between the two rivers regarding their seasonal temperature variation pattern, which occur mostly in the end of spring and summer when the NRR presents significantly higher average daily temperatures. Flow regulation caused by Vilarinho das Furnas operation seems to have no substancial effects on the expected annual thermal pattern of the RR since a strong correlation (Spearman rank correlation; rho > 0.90, P-value < 0.05) exists between mean monthly temperature values of this river and the respective local air temperature registered in the closest meteorological station (SNIRH, 2013).

Regarding streamflow regime, the regulation for storage and derivation purposes in the RR is affecting its flow regime by reducing and homogenizing the magnitude of the annual and monthly volumes and variability of habitual and extreme flow values throughout the year, when compared with the NRR river (Figure 1). The mean annual flow volume of the NRR is 371.59 hm^3, much higher than the value observed for the RR (82.94 hm^3). The difference between the maximum and the minimum flow volumes along the year is higher in the NRR (99.36 hm^3) than in RR (18.89 hm^3). This type of regulation is also affecting, in a particular way, the flood season by severely reducing the frequency, duration and magnitude of the maximum daily flow volume registered (NRR: 224.30 hm^3; RR: 43.74 hm^3), the effective discharge (flood with power to change the geomorphology of the river; NRR: 210.30 hm^3; RR: 58.88 hm^3) and the variability of floods, among others. The drought season is significantly less affected by this type of regulation, and only a small reduction on the average of the minimum flow volumes during the drought season is observed in the RR (1.57 hm^3), when compared with the NRR (4.8 hm^3).

This streamflow characterization was performed using IARHIS 2.2, a methodology developed by Santa-María and Yuste (2010), which proposes a set of Indicators of Hydrologic Alteration (IHA) and compares them between non-regulated and regulated rivers to evaluate deviations caused by river regulation to the most environmentally important components of the flow regime.

2.2. COLLECTION OF FISH DATA

In each selected river, fish samples were taken from three distinct sites (Figure 1). Sampling sites in the non-regulated (River Vez; NRR) and in the regulated (River Homem; RR) rivers were equally distributed (~2 km from each other). In the regulated river, sites were located downstream of the dam (≥3 km) but before the entry of any major tributary to avoid the significant amelioration of regulation effects in flow regime. Fish samples were taken from three independent sites only to increase population representativeness and these sites were never analysed as independent samples to avoid pseudo-replication issues. For replication purposes individual fish/river combinations were considered.

An electrofishing (Hans Grassl EL 62 generator DC, 600 V) samping method was used to collect bi-monthly samples of Northern Iberian chubs between May 2010 and June 2011. During the reproductive period of the target species theroretically described in the literature (March-June; Maia *et al.*, 2006), sampling was fortnightly. In total, 321 chubs were caught in the studied rivers (River Vez: 183; River Homem: 138). All fish samples were immediately placed on ice until they were stored at -10ºC in the laboratory, where their total (L_t, by the longer caudal fin lobe and to the nearest mm) length and weight (W_t, ± 0.01 g) were measured. After the complete removal of the viscera, fish were again weighed (eviscerated body mass, W_e, ± 0.01 g). Gonads were removed, visually inspected for sex determination (males, females or immature) and their weight (W_g, ± 0.01 g) was determined. Gonads from female chubs captured in the two studied rivers (Vez: n=22; Homem: 19) during the theoretical peak of their reproductive cycle (i.e., May and June 2011; e.g., Maia *et al.*, 2006) were weighed and placed on a 4% solution of neutralized formaldehyde and, after three days, washed with distilled water and preserved in 96% ethanol for the analysis of fecundity and oocyte size distribution.

From different sections of each pair of preserved ovaries, five sub-samples of approximately similar weight were weighed and stored in alcohol. Sub-samples were shaken periodically to aid oocyte separation. The total number of oocytes in each gonadal sub-sample was counted for fecundity determination, while 100 oocytes from each sub-sample were also measured for size distribution analysis (± 0.001 mm), using a digital camera (LEICA DFC 280), coupled to a stereomicroscope (LEICA MZ6), and the image analysis program LEICA Application Suite 4.1.0 (LEICA Microsystems).

From each sampled chub, 20 scales from the left side of the body, between the dorsal fin and lateral line, were removed for age determination. Scales were cleaned using a 10% NaOH solution, dried, mounted between two glass slides and projected under constant magnification (20×). The best scale of each fish was chosen and all the measurements were made on it. The number of annulus (fish age) was counted and the total scale radius, as well as the distance from the focus to each annulus, was measured on the lateral-ventral field of each fish scale.

2.3. AGE AND GROWTH

The relationship between scale radius and fish total length was fitted for each of the studied chub populations using a linear regression model. Since estimated intercepts (constant *a*) were significant for the two modelled relationships ($p<0.05$), we rejected the null hypothesis that these intercepts were not different from 0 and included them in the respective age-length back-calculations as an estimate of the Fraser-Lee correction factor (considered as the fish length at scale formation). Back-calculations were performed separately for each population using the Fraser-Lee equation (Bagenal & Tesch, 1978):

$$L_{\mathrm{i}} = \frac{L_{\mathrm{t}} - a}{S_{\mathrm{c}}} \times S_{\mathrm{i}} + a,$$

where L_i is the length at annulus formation, L_t is the total fish length at capture, S_i is the radius at annulus formation, S_c is the overall radius and *a* is the regression intercept or the size of the individual at the time of scale formation determined for each population. SEX and POPULATION/RIVER effects in the back-calculated lengths-at-age were evaluated by an analysis of covariance (ANCOVA), to test the null hypothesis of no differences between rivers or sexes in the slopes of length-at-age regressions.

Annual increments and instantaneous growth rates (Bagenal & Tesch, 1978) were obtained from back-calculated lengths. POPULATION/RIVER and SEX effects in fish back-calculated annual increments were tested using an ANCOVA, to test the null hypothesis of no differences between rivers or sexes in chub's growth. It is a well known fact that fish growth rate is intimately related with fish age and size, since this parameter usually declines when fish get older and bigger (Bagenal & Tesch, 1978). Therefore, to account for and, if necessary, correct age/size effect in these comparisons, we included estimated fish length-at-age as covariate, as well as its interaction with main tested factors (POPULATION/RIVER and SEX), in the ANCOVA analysis. This analysis was followed by Tukey HSD post-hoc tests to identify significantly different levels within the tested factors.

Periodicity of annulus deposition, and consequently, scale reading procedure and subsequent analysis, were validated by marginal increment analysis (MIA; Bagenal & Tesch, 1978), defined as:

$$MIA = \frac{S_{\mathrm{c}} - Y_{\mathrm{i}}}{Y_{\mathrm{i}} - Y_{\mathrm{i-1}}},$$

where Y_{i} is the radius of the last annulus, $Y_{\mathrm{i-1}}$ is the radius of the penultimate annulus and S_{c} has the same meaning as before. Mean MIA values (± standard deviation) for each sampling campaign, together with the instantaneous increment rates between sampling campaigns, were used to analyse seasonal growth of the two studied populations. Significance of intra-annual variations of MIA was evaluated by a one-way analysis of variance (ANOVA), using MONTH as the single fixed factor, followed by Tukey HSD post-hoc tests to identify significant growth periods.

Observed fish lengths, and respective ages, at capture were used to determine Von-Bertalanffy growth equations (Ricker, 1975) for the two studied populations, as following:

$$L_t = L_\infty(1 - e^{-K(t-t_0)}),$$

where, L_{t} is the length at time t, L_∞ is the asymptotic length, K is a growth coefficient and t_0 is a time coefficient at which length would theoretically be 0. Von-Bertalanffy analyses were conducted separately for males and females of each population, and immature fish were included in each sex for both procedures. Equations were fitted and parameters were estimated using the "Analysis of Length-at-age data" option included within the package FISAT II – Fish Stock Assessment Tools, v1.2.2., developed by FAO-ICLARM (2005).

Weight-length relationship was also analysed and compared between the two studied populations. As for Von-Bertalanffy estimates, in these analyses immature chubs were also included in each sex in the following procedures. Length and weight values were log-transformed to achieve data linearity and the models were fitted using linear regression procedures, as the following equation:

$$\log(W_{\mathrm{t}}) = \log(a) + b \times \log(L_{\mathrm{t}}),$$

where b and $\log(a)$ are, respective, the slope and the intercept of the relationship, and W_{t} and L_{t} represent the same as before. Inferences about the slope of each linear model, representing the type of growth exhibited by the fish, were performed using a *t*-test of mean difference against a constant value of 3 (isometric growth). Comparisons of weight-length relationship between chubs' POPULATION/RIVER and SEX were performed using an ANCOVA.

2.4. REPRODUCTION

Age and length at first maturation were determined separately for male and female chubs from the non-regulated and regulated rivers following two main criteria:

i) age and total length of the youngest and smallest fish exhibiting gonads on stages III, IV, V or VI of maturation (Murua *et al.*, 2003);

ii) age-class, and respective mean total length, in which at least 10% of the fish exhibit gonads on stages III, IV, V or VI of maturation (Murua *et al.*, 2003).

Gonadal development cycle was assessed separately for male and female chubs captured in each river/campaign combination using the Gonadossomatic Index (*GSI*):

$$GSI = \frac{W_{\mathrm{g}}}{W_{\mathrm{e}}} \times 100.$$

T-test analyses were used to statistically compare this index between the two studied populations in each sampling campaign.

Univariate and multivariate one-way PERMANOVA (add-on package PERMANOVA for PRIMER+v6.0; Anderson *et al.*, 2008) showed the absence of significant differences in, respectively, oocyte number and size frequency distribution (0.1 mm size classes, between 0 and 2.4 mm), regarding their position in the ovary, so all five sub-samples taken from each fish were used in the subsequent analyses. Fecundity was determined for each individual female, using the gravimetric method, as the product of mean oocyte density and gonad weight, as the following equation:

$$F = \frac{(\sum_i \frac{O_\mathrm{i}}{W_\mathrm{i}})}{n} \times W_\mathrm{g},$$

where, Oi is the total number of oocytes in a sub-sample, Wi is the weight of the respective sub-sample, n is the number of sub-samples taken (i.e., five) and Wg is the same as before. Differences in fecundity and oocyte size distribution between the two studied chub populations were tested by means of, respectively, univariate and multivariate one-way PERMANOVA, considering POPULATION/RIVER as the only fixed factor and fecundity and oocyte size classes as dependent variables. Oocyte size-classes occurring in less than 5% of the samples were removed. Some studies on cyprinids (e.g., Herrera & Fernández-Delgado, 1992; Fernández-Delgado & Herrera, 1995; Maia *et al.*, 2006) reveal a strong relationship of fecundity and oocyte size with fish length, therefore we included individual total length (L_t) of analysed females as a covariate to test its effect on the variability of these two parameters.

2.5. RELATIONSHIP WITH ENVIRONMENTAL VARIABLES

The hydrological characterization presented before in this manuscript, which was performed with IAHRIS 2.2, plus a Time Series Analysis (TSA) (River Analysis Package version 1.3.0; Marsh *et al.*, 2006) on the same flow time series for both rivers, allowed us to obtain a total of 27 monthly (January to December) hydrologic metrics, all of them with potential to be representative of the degree of hydrological alteration caused by the river regulation source (i.e., Vilarinho das Furnas Dam) analysed in this study. Initial screening (Spearman rank correlation, rho) showed strong correlation among many of these metrics (above a cut-off value of 0.80). To avoid redundancy among the tested hydrological predictors, when two or more variables were considered highly correlated, only one of them was selected, usually the most ecologically relevant. Following this selection procedure, from the initial set of potential predictors, 10 final hydrological metrics (Table 1) were selected, describing ecologically important aspects of the flow regime and its alterations in the studied systems.

Besides river hydrology, thermal regime and associated ecological cues are also considered of high importance for the development of fish life-cycles and, at the same time, are some of the main environmental factors usually affected by the general operation of dams, independently of the purpose these infra-structures were built for (Olden & Naiman, 2010). Considering this, we added an eleventh environmental variable to the list of potential predictors, the mean monthly temperature (*Temp*), to evaluate its joint effect with streamflow components in the intra-annual variation of chub's life-cycle parameters.

Table 1. Streamflow and temperature variables, and respective range of values for each studied river during the entire study period (mean monthly values), selected to assess the relationship between the environmental variability within the study area and chub's reproductive and growth seasonal cycles

Streamflow variables (unit)	*Code*	Non-regulated river (River Vez; NRR)	Regulated river (River Homem; RR)
Monthly volume (hm^3)	*MonthVol*	2.56 - 61.72	1.65 – 10.75
N_o of high flow days (days)	*HFlowDays*	0.00 – 2.20	0.00 - 0.00
Flow variability ($Q_{10\%}$-$Q_{90\%}$)	*Var*	1.20 – 2.49	0.47 – 1.67
N_o of null flow days (days)	*ZFlowDays*	0.00 – 4.58	0.00 – 0.00
Duration of high spell peaks (days)	*DHSpelPeak*	0.00 – 3.54	0.00 – 8.27
Magnitude of low spell troughs (m.s$^{-3)}$	*LSpelTrough*	0.00 – 0.39	0.00 – 0.53
Duration of low spell troughs (days)	*DLSpelTrough*	0.00 – 17.76	0.00 – 12.27
Period between low spells (days)	*PBLSpel*	0.00 – 13.00	0.00 – 6.50
Duration of falls (days)	*DFalls*	6.32 – 13.89	4.97 – 9.31
Baseflow (m.s^{-3})	*BsFlow*	0.36 – 0.78	0.62 – 0.84
Mean monthly temperature (ºC)	*Temp*	9.95 - 22.20	10.48 – 20.33

Stepwise multiple linear regressions (*P-value* to enter = 0.05; *P-value* to remove = 0.10) were used to relate the 11 final environmental variables with chub's annual reproductive cycle, expressed as the gonadossomatic index (*GSI*), and seasonal growth, expressed as instantaneous MIA increments between sampling campaigns. For seasonal growth analyses, the period in which the new annulus was deposited was ruled out, since the accentuated decrease in marginal width cannot be considered an alteration of fish growth pattern (Bagenal & Tesch, 1978).

For all the statistical analyses described in this study, assumptions for the use of appropriate parametric methodologies were previously tested and, in case of non-fulfilment even after suitable data transformation, the equivalent non-parametric analysis was employed. With the exception of PERMANOVA, all statistical analyses were conducted with STATISTICA 13.0 (StatSoft, Inc.)

3. RESULTS

3.1. AGE AND GROWTH

Marginal increment analysis (MIA) validated the annual deposition of the annulus in chubs' scales for the two studied populations. Annulus formation occurred in the beginning of May for the two populations and sexes (Figure 2). Male chubs inhabiting the NRR presented a total of six age groups while females from the same population exhibited seven age groups. Seven age groups were also found for male and female chubs inhabiting the RR.

The ANCOVA performed on the back-calculated length-at-ages obtained for the two populations and sexes revealed significant effects of SEX ($F_{2,\ 901}$ = 7.23; p<0.001) but not POPULATION/RIVER ($F_{1,\ 901}$ = 1.33; p>0.05), on the length-at-age relationship of tested chubs. Besides this, only the interaction term of SEX with the covariate AGE (i.e., back-calculated annulus increment number) was significant ($F_{1,\ 901}$ = 24.22; p<0.001), revealing that the slopes of the tested regressions were only significantly different between male and female chubs and not between NRR and RR populations. Therefore, we chose to present length-at-age data separately for male and female chubs within each studied river (Table 2) and to perform following growth analysis and comparisons independently for each sex.

The ANCOVA performed to identify POPULATION/RIVER and SEX effects on chub annual growth (i.e., back-calculated annual increments) identified a significant effect of the covariate length-at-age ($F_{1,\ 580}$ = 10.71; p<0.05), confirming the strong relationship between chub's growth and respective age/size. This analysis also identified a significant effect of SEX ($F_{2,\ 580}$ = 5.15; p<0.05), but not of POPULATION/RIVER ($\mathrm{F}_{1,\ 580}$ = 2.66; p>0.05) on chubs' annual growth. None of the tested interactions, between factors or with covariate, had significant effects on fish growth. Additional Tukey-HSD tests on the levels of significant factor SEX showed that female chubs exhibit a higher annual growth when compared with males, independently of the river/population they come from.

Table 2. Back-calculated total lengths (mean total length, mm) for male and female chubs from the two studied populations/rivers (NRR and RR), with data from another study with the same species (Maia *et al*., 2006[1]; mean fork length, mm) given for comparison

	I		II		III		IV		V		VI		VII
Studied populations													
River Vez (NRR) - Males	52		71		92		112		122		131		
Annual increment		19		21		20		10		9			
Instantaneous growth rate		0.31		0.26		0.20		0.08		0.07			
River Vez (NRR) - Females	52		73		95		120		144		164		183
Annual increment		21		22		25		24		20		19	
Instantaneous growth rate		0.34		0.26		0.23		0.18		0.13		0.11	
River Homem (RR) - Males	49		67		86		106		124		141		158
Annual increment		18		19		20		18		17		17	
Instantaneous growth rate		0.31		0.25		0.21		0.16		0.13		0.11	
River Homem (NRR) - Females	51		72		93		119		147		169		196
Annual increment		21		21		26		28		22		27	
Instantaneous growth rate		0.34		0.25		0.25		0.21		0.14		0.15	
Other studies													
River Estorãos[1] – Combined sexes/Fork length	45		69		95		116		135				
Annual increment		24		26		21		19					
Instantaneous growth rate		0.43		0.32		0.20		0.15					

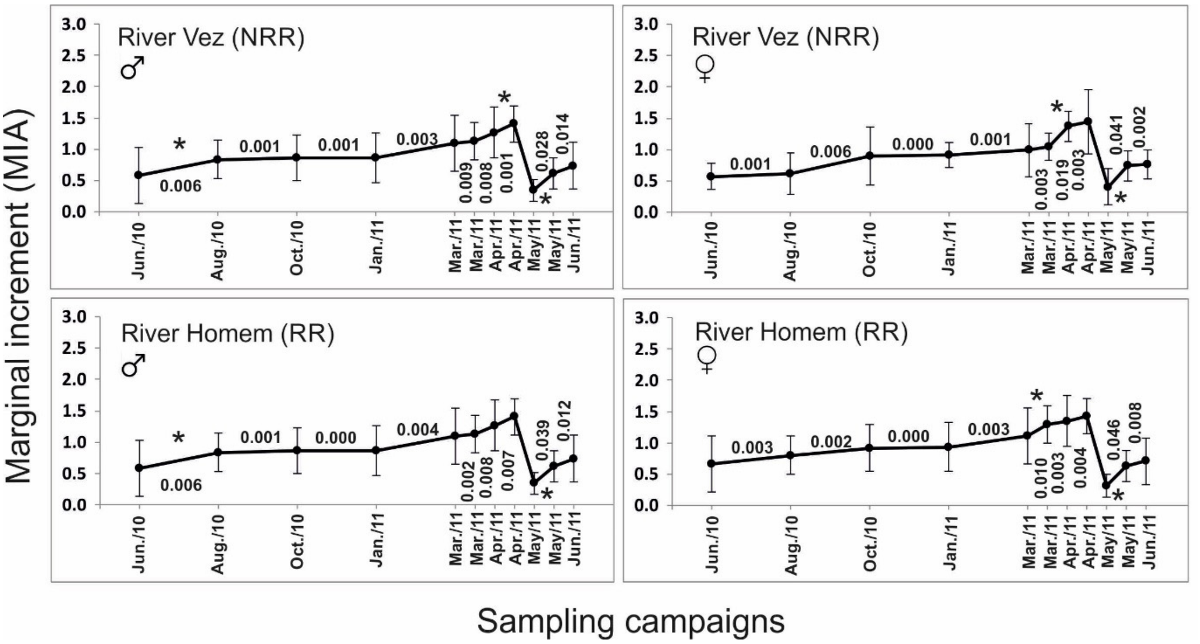

Figure 2. Seasonal variation of marginal increments (MIA; mean ± standard deviation), obtained from the scales of male (♂) and female (♀) chubs from the two studied populations/rivers. Instantaneous increment rates between sampling campaigns are also indicated and * shows statistically significant intra-annual variations of MIA (ANOVA; *p*<0.05; TukeyHSD tests).

Von-Bertalanffy growth equations and parameters estimated for each population using observed fish lengths, and respective ages, at capture corroborated the results previously obtained from the ANCOVA of back-calculated annual increments, showing that differences in growth rate (*k*) and maximum potential length (asymptotic length, L_∞) were higher between sexes than between populations/rivers studied. In accordance with the equations and parameters presented below (i.e., parameters of interest signaled in bold), within both populations, independently of the level of flow regulation they were subjected to, female chubs showed higher growth rates and have the potential to achieve larger sizes than males.

$$NRR\ Males\ (n = 142) \rightarrow L_{\mathrm{t}} = \mathbf{184.7}(1 - e^{-\mathbf{0.30}(t)})$$

$$NRR\ Females\ (n = 127) \rightarrow L_{\mathrm{t}} = \mathbf{251.8}(1 - e^{-\mathbf{0.35}(t)})$$

$$RR\ Males\ (n = 94) \rightarrow L_{\mathrm{t}} = \mathbf{170.1}(1 - e^{-\mathbf{0.34}(t)})$$

$$RR\ Females\ (n = 89) \rightarrow L_{\mathrm{t}} = \mathbf{284.6}(1 - e^{-\mathbf{0.37}(t)})$$

Seasonal growth analysis, based on the instantaneous MIA increments, revealed, once again, that differences in growth patterns, this time analysed at a reduced temporal scale (i.e., seasonal), are mostly between sexes rather than between chub populations subjected, or not, to flow regulation (Figure 2). Even between sexes, differences in seasonal growth pattern are slightly noticeable and may reflect only small gender-related anticipations and/or delays constrained by the respective peak of reproductive season. In general, in terms of seasonal growth chubs from both sexes exhibited two distinct periods. A period of significant growth during spring, between March and June, which is coincident with the species reproductive season, and a second period of reduced, or indistiguishable growth during the end of summer, autumn and winter. The period of accentuated and statistically significant growth (ANOVA; $p<0.05$; *) during spring seems to start earlier (i.e., ca. one month) in females than in males, for both rivers. Multiple regressions revealed that seasonal MIA increments were not significantly related to any of the environmental variables used in these analyses as potential predictors for this biological cycle (Table 3), implying that seasonal growth of male and female chubs is poorly responsive to environmental variation, or, is somehow related to other variables not included in this study.

Table 3. Model summary of multiple regression analyses between growth and reproductive seasonal cycles of male and female chubs and environmental variables related with streamflow and temperature. Statistically significant values are highlighted in bold

Variables	Regression coefficients	s.e.	*t*
Seasonal growth (*Instantaneous MIA*)			
Males			
No variables were selected by the model			
Females			
No variables were selected by the model			
Gonadossomatic index (GSI)			
Males			
No variables were selected by the model			
Females			
[Total adjusted $R^2 = 0.72$; $F_{2,19} = \mathbf{10.35}$]			
DFalls	0.76	0.22	**3.43**
Var	-8.49	2.77	**-3.06**
Females GSI = 9.28 + 0.76*DFalls* - 8.49*Var*			

Regarding the log-transformed (i.e., linear) weight-length relationship for males and females of the studied populations (Table 4; Figure 3 for graphic representation of linear weight-length relationships for both populations/rivers), t-tests between the estimated slopes and the constant 3 (defined as isometric fish growth) revealed the existence of a significantly different type of growth between the two studied populations, which was consistent for both sexes. While male and female chubs from NRR exhibited a growth type statistically similar to isometric growth, chubs from the RR exhibited a significant positive allometric growth type (t-test; $p<0.05$), where fish tend to become "plumper" as they increase in length.

Table 4. Linear weight-length regression coefficients $[\log(W_t) = \log(a) + b \times \log(L_t)]$ estimated for male and female chubs in the two studied systems. Immature chubs were included in both sexes. Slopes (b) significantly different (t-test; *p-value*<0.05) from 3 (isometric growth) are highlighted in bold

Population/River	Sex	no. of fish	log (*a*)	*b*	*adjusted* R^2
River Vez (NRR)	♂	142	-11.53	2.99	0.96
	♀	127	-11.60	3.03	0.98
River Homem (RR)	♂	94	-12.42	**3.19**	0.98
	♀	89	-12.51	**3.21**	0.99

ANCOVA on log-transformed weight-length relationships revealed significant effects of POPULATION/RIVER ($F_{1,\,441}$ = 10.66; $p<0.05$), and of its interaction with the covariate, log-transformed L_t ($F_{1,\,441}$ = 14.26; $p<0.001$), on the linear regressions between log-transformed lengths and weights of tested chubs. The factor SEX ($F_{1,\,441}$ = 0.55; $p>0.05$), as well as its interactions with L_t ($F_{1,\,441}$ = 0.17; $p>0.05$) and POPULATION/RIVER ($F_{1,\,441}$ = 0.66; $p>0.05$) did not had a significant effect on this relationship. These results indicate that length-weight relationship is different between the two studied populations, but this variation is dependent of fish ontogeny. Smaller fish have higher condition in NRR but, as they grow, adult chubs become plumper and present higher condition in RR (Figure 3).

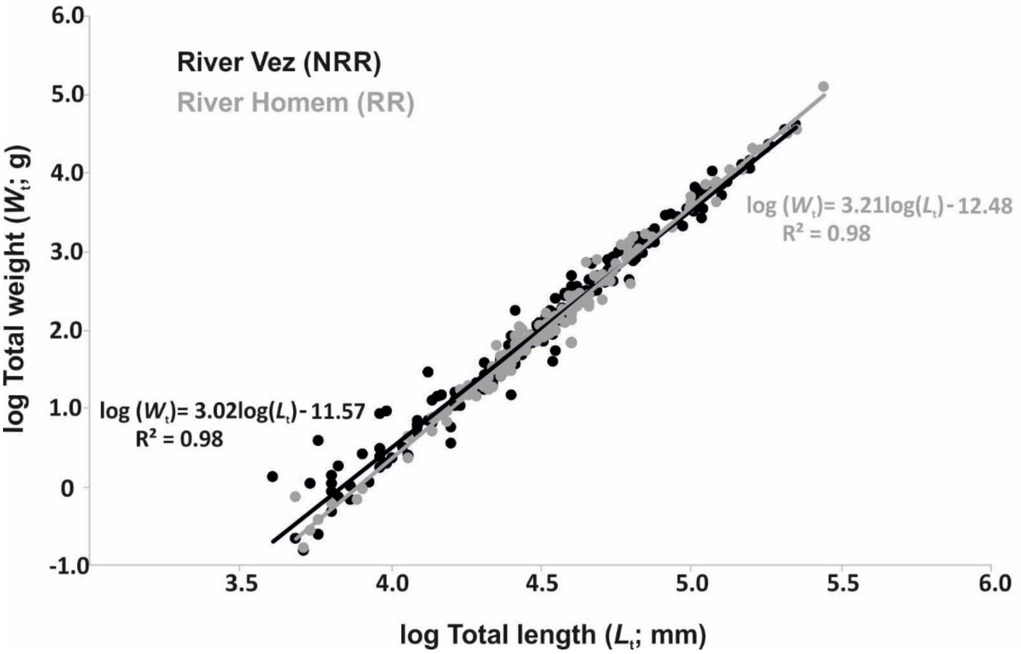

Figure 3. Linear weight-length relationships for chubs from the two studied rivers (NRR and RR) and respective fitted linear equations. Immature chubs were included in both sexes.

3.2. REPRODUCTION

In this study, age and length at first maturation for studied chub populations from non-regulated and regulated watercourses was determined following two previously described criteria. Per the first criteria, age at first maturation was similar between both rivers and was only different between males and females. Male chubs reached maturity at 2 years in both rivers and at a similar minimum size (NRR: 60 mm; RR: 69 mm). Female chubs reached their maturity a year later in both rivers, with 3 years, and at similar minimum sizes (NRR: 94 mm; RR: 90 mm). The second criterion used to assess these biological traits confirmed these results and the observed mean total lengths of the age-classes at which the first maturation occurred were the following: NRR Males: 72 mm (2^+); NRR Females: 94 mm (3^+); RR Males: 74 mm (2^+); RR Females: 92 mm (3^+).

Regarding gonad activity (GSI), male chubs presented a similar intra-annual pattern of variation between NRR and RR, with small differences being observed only for the respective peak of the reproductive season (Figure 4). Males inhabiting RR exhibited a shorter duration of this period, reaching its peak in the beginning of May and finishing it almost immediately, whereas NRR chubs prolonged it during the entire month. In short, male chubs from both rivers presented three clearly defined phases: quiescence (August to March), gonad maturation (March to May) and reproduction (May).

For female chubs (Figure 4), differences in gonad maturation pattern were more evident than for males, especially during their reproductive season. As for males, female chubs from both rivers also presented the three annual phases, namely: quiescence (August to March), gonad maturation (March to May or June, depending on the population) and reproduction (May or June, depending on the population). However, NRR female chubs had a slower period of gonad maturation that reached its peak only in the beginning of June coupled with a shorter spawning period, while females from RR matured quickly and reached their peak of gonad maturation, and respective spawning, a month earlier, in May, and prolonged it until June.

Multiple regressions performed to identify the main environmental predictors of gonad maturation level (GSI) for male and female chubs from both studied rivers presented distinct results for each sex (Table 3). None of the proposed environmental variables was selected as being significantly related with male GSI, but for females the applied analysis identified *DFalls* and *Var* as being, respectively, positively and negatively related with the temporal variation of this biological trait.

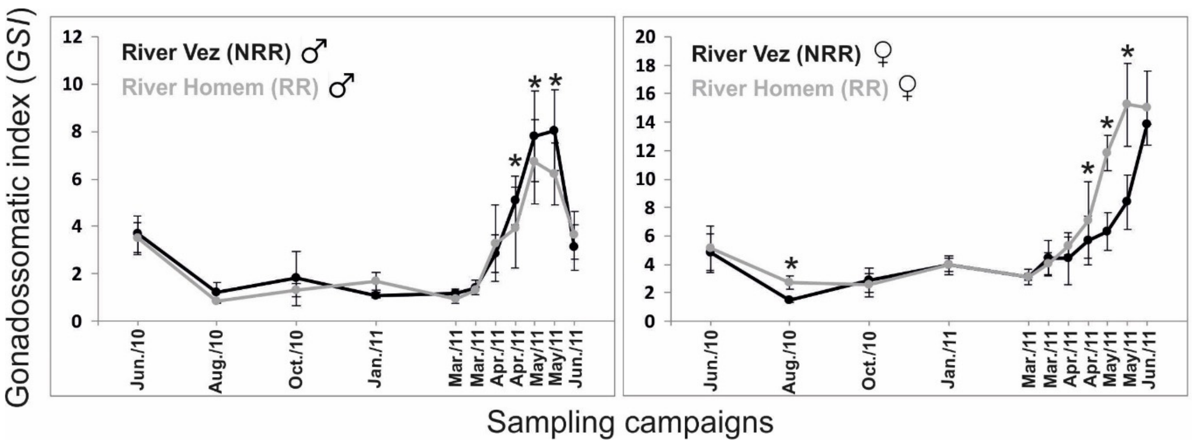

Figure 4. Seasonal variation of the gonadossomatic index (*GSI*; mean ± standard deviation) for male (♂) and female (♀) chubs from the two studied populations/rivers. Significant differences of *GSI* values are indicated for the respective river/sampling seasons (*t*-tests; $p<0.05$).

The PERMANOVA performed to test differences in female chubs' fecundity between both studied rivers identified a significant effect of fish length in the analysed samples ($F_{1,83}$ = 37.34; $p<0.05$). The factor POPULATION/RIVER ($F_{1,39}$ = 7.54; $p<0.05$), but not its interaction with covariate, had significant effects on chub fecundity indicating that, independently from their size, female chubs from NRR (*ca*. 5200 eggs in average) had a higher fecundity than females from RR (*ca*. 3000 eggs in average).

PERMANOVA performed to test differences in oocyte size distribution between female chubs from both studied populations also identified a significant effect of the covariate (i.e., fish length) on the analysed samples ($F_{1,203}$ = 5.38; $p<0.05$). While controlling for this covariate, POPULATION/RIVER ($F_{1,203}$ = 5.33; $p<0.05$) showed significant effects on oocyte distribution. The results of the PERMANOVA coupled with the oocyte size distribution presented in Figure 5, show that larger eggs are more common in RR population, associated to only one peak of distribution, while females from NRR have a second batch of smaller eggs.

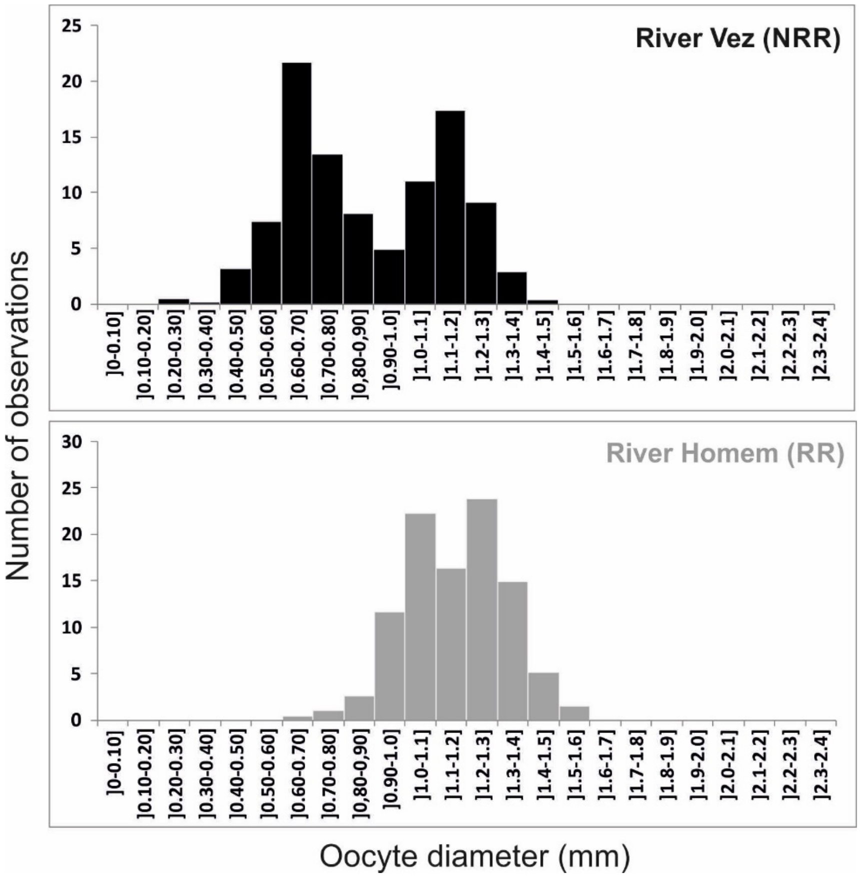

Figure 5. Size-frequency distribution of oocytes (mean number of observations per size class, considering all sub-samples analysed) from female chubs caught in the two studied populations/rivers during the peak of the respective reproductive period (May and June 2011).

4. DISCUSSION

Although being a widely distributed species, especially in north-western Iberia, which includes the Minho, Lima, Cávado, Douro, Vouga and Mondego river basins (Carmona & Doadrio, 2000), only a small number of bio-ecological studies are available for Northern Iberian chub, dealing with parameters such as density, biomass, age, growth and reproductive patterns (e.g., Valente, 1993; Santos *et al.*, 2004; Maia *et al.*, 2006). Therefore, the results obtained in this study can contribute to increase knowledge about this taxon and how it responds to environmental variation caused by flow regulation, coupled with EFR implementation, which can help to develop appropriate management strategies for this, or other more threatened, small resident fish species.

4.1. FLOW REGULATION EFFECTS

Overall, the results obtained in the present study suggest that most life-history characteristics of the target species did not present a significant change when facing the studied streamflow regulation scenario, not differing substantially between the two studied populations and from those found for other Iberian *S. carolitertii* populations (e.g., Valente, 1993; Maia *et al.*, 2006), as well as for its sister species, *Squalius pyrenaicus* (Günther, 1868), populations (e.g., Magalhães, 1993; Fernández-Delgado & Herrera, 1995). In general, we identified a high degree of segregation between life-cycle patterns of male and female chubs, which was already an expected result since this is relatively common for most species of cyprinids (Mann, 1991). Regarding life-history differences between non-regulated and regulated rivers, only some of the analysed traits of chub populations seemed to be affected and altered by the flow and habitat homogenization that the derivation dam, and its EFR, are causing on the impounded river.

Weight-length relationship, considered as one of the most appropriate measures of fish condition (Przybylski *et al.*, 2004), was one of the major differences described in this study between the two populations. We found a significant effect of population/river on the type of growth presented by the studied chubs, but, since fish length influenced the obtained relationships, the described pattern is not constant throughout all chub ontogeny. In short, smaller fish have better condition in NRR but, as they grow, adults become “plumper” and present higher condition in RR. Also, the NRR population exhibited a “isometric” growth type, which is common to other populations of the species (Valente, 1993; Maia *et al*., 2006), while RR chubs presented a "positive allometric" growth, revealing an abnormal pattern which can be assumed as a response to the environmental alteration imposed by the studied dam. Patterns of weight-length relationship and associated insights on fish body condition, usually reflect a combination of two driving forces acting together with environmental variability. On one hand, the described pattern can be related with the link between chub's dietary preferences, and its ontogenic variability, and the availability of food resources in both streamflow scenarios. The northern Iberian chub, as well as other species of this genera, is generally described as an omnivorous species, feeding on both animal and plant items, with a strong ontogenic variation on its dietary habits (e.g., Magalhães, 1993; Garrido *et al*., 2003; Sanchez-Hernandez & Cobo, 2012). As described by these authors, smaller chubs tend to feed more on small soft-bodied invertebrates, which in this case are more available at the NRR river (Alexandre *et al*., 2015b), contributing for the higher condition of these fish in the non-regulated watercourse. As they grow, chubs tend to decrease animal prey breadth and feed more on larger items, such as smaller Decapoda and detritus/plant materials (Magalhães, 1993; Garrido *et al*., 2003), whose constantly higher abundance and availability is incremented in the studied regulated river by the environmental homogenisation caused by its derivation dam (Alexandre *et al*., 2015b), contributing to the higher body condition of adult chubs in RR. The other driving force suggested as taking part on the described link between environmental variability and chubs condition is fitness. In general, a lower body condition, like it was observed for adult chubs inhabiting the NRR, is associated to a more swim-fitted morphology to deal with high, more irregular and turbulent flows (Alexandre *et al*., 2014b). Smaller, juvenile, chubs, may not yet present so clear this phenotypical response but, as they grow and phenotype starts to be modelled by the environment, the distinct habitat conditions to which fish are subjected in the two studied systems cause the observed eco-morphological differences, with chubs from NRR presenting a lower body condition and chubs from RR showing a higher condition in response to an artificially homogenised environment, in which high flows are reduced, habitat is more stable and food is constantly available.

Besides condition, some reproductive traits of chubs, particularly from females, also exhibited significant differences between the two studied rivers, which can be attributed to flow regulation caused by the derivation dam and the implemented EFR. Female chubs inhabiting RR presented a quicker maturation and extended spawning periods than females from NRR. Fish from the regulated river also presented a reduced fecundity and a single batch of larger eggs when compared with the females from the non-regulated river, for which a higher fecundity divided by two batches of eggs was observed. Patterns observed for male chubs from NRR and RR and females from NRR were like what is generally described for this, and other congener, species regarding reproductive traits (Fernández-Delgado & Herrera, 1995; Maia *et al*., 2006). However, significantly different patterns were observed for RR females, which again may be related with the environmental artificialization imposed by the derivation dam on the studied regulated river.

Reproductive cycles of freshwater fish are strongly dependent of the simultaneous occurrence of a set of environmental predictors that provide the optimal conditions for fish to mature and spawn (Humphries *et al*., 1999). In this study, an increase in gonad activity of female chubs was related with two streamflow variables associated with periods of higher stability of the intra-annual discharge pattern, namely an increase in the duration of flow falls (*DFalls*), and a decrease of flow variability (*Var*). Reproduction of this species occurred when optimal conditions of these variables occurred simultaneously, but intra-specific differences on reproductive traits of female chubs between non-regulated and regulated rivers accompanied the specific environmental variability of each of the studied systems. For fish inhabiting northern permanent non-regulated rivers, such as NRR, the end of spring, for example in May, may still not have the ideal conditions for chub's reproduction, as flow variability is often considerably high. In RR river, the derivation dam causes a decrease of typical spring peak flows and associated environmental variability, allowing female chubs to anticipate the maturation and spawning periods. Also, the higher body condition of adult fish in RR may promote this patterns since the high levels of energy reserves can induce an anticipated and prolonged reproduction (Moyle & Cech, 1996). Differences obtained for fecundity and oocyte size between the two chub populations, and the relationship of these traits with environmental variability and flow regulation, can be debated at the light of the triangular model of life-history evolution proposed by Winemiller and Rose (1992), which discusses fish life-history strategies as being adaptive with respect to variability, predictability and seasonality of streamflow regimes (Winemiller, 2005). Within this context, differences of fecundity and oocyte size distribution between the two populations can be discussed as an adaptive response to the specific characteristics of the two flow regimes. Female chubs inhabiting the NRR are subjected to a more variable and unpredictable environment and tend to maximize their reproductive success by producing a large quantity of eggs and releasing them in several batches (i.e., in this case two), similar to what has been described for other cyprinid species has having several advantages for egg and juvenile survival (e.g., Herrera & Fernández-Delgado, 1992; Fernández-Delgado & Herrera, 1995; Torralva *et al*., 1997; Durham & Wilde, 2009; Alexandre *et al*., 2015a). In opposition, fish from RR face a more constant and predictable environment, with less prominence to egg and juvenile mortality, so the investment they do on the number of eggs and batches released, is also reduced.

4.2. MIGRATORY VS RESIDENT SPECIES

Annual variation in the hydrograph should affect species with distinct life-history strategies differently, thus determining variation in the composition and structure of fish assemblages (Agostinho *et al*., 2004). Considering this, the responses obtained in this study for the target non-migratory species, the Iberian chub, to the studied flow regulation scenario, can be compared with the results previously obtained (Alexandre *et al*., 2015a) for a larger and more mobile fish species, the Iberian barbel, and provide novel insights about the way how different fish species cope with imposed environmental conditions.

In general, (check Table 5 for a summary of the different responses exhibited by both species to the same type of flow regulation), life-history traits of the target non-migratory species were similar between the two rivers and the only significant responses to artificial environmental homogeneity imposed by the derivation dam were reflected mostly by female fish through changes in the duration of spawning period, fecundity and oocyte size distribution. Only fish condition showed a response to flow regulation for both sexes. In opposition, flow regulation promoted wider responses from the migratory species, most of them common to males and females, reflected by an altered seasonal growth pattern, a decrease of fish growth rates, body condition and gonad activity, and an increase of maximum length and longevity. The two-species presented contradictory results when facing the same type of flow regulation, which proves the complexity of the existing relationships between fish biological cycles, life history strategies and environmental variability (e.g., Mims & Olden, 2012; 2013; Alexandre *et al*., 2015a). However, considering the results obtained for the present and previous studies, the migratory species seem to exhibit a high degree of change in their biological cycles, both in terms of growth and reproduction, in relation to artificiallyinduced environmental homogenisation than the studied resident species. This is probably related with the specific life-history strategies and cycles, and associated cues and habitat requirements of both types of species.

In general, migratory and high mobile species, like the potamodromous Iberian barbel, have a high reliance on habitat and environmental features to complete their different life-history processes (Lucas & Baras, 2001; Lennox *et al*., 2016). Ultimately, at specific stages or periods of their annual and seasonal cycle (e.g., migration, growth, reproduction), they tend to display reophilic behaviour and/or present demographic strategies particularly mediated by the timing and intensity of flow and temperature cues (Pavlov *et al*., 2008; Skov *et al*., 2010), variables that are usually less important (i.e., confirmed by the poor relationships obtained in this study between chub's seasonal growth/GSI and environmental predictors) for the development of the more flexible and generalist life-cycle strategies of resident species (Lucas & Baras, 2001; Tedesco *et al*., 2008). These aspects make migratory fish more susceptible and responsive than non-migratory ones to environmental changes caused by river regulation, and they often show a higher biological deviation when subjected to the environmental differences that exist between natural and regulated flow regimes (Naiman *et al*., 2008), similarly to what was observed in the comparison presented in this study. Most flow regulation modes, but especially EFR regimes, tend to homogenise downstream habitats and environmental conditions, with loss of the typical fluvial variability and reduction or alteration of environmental cues that migratory fish require for the development of most of their ecological processes, such as migration, growth or reproduction (Araújo *et al*., 2013).

Table 5. Comparison of life-cycle traits' response to studied flow regulation scenario, between the target migratory (Iberian barbel; Alexandre *et al*., 2015a) and resident (Iberian chub; present study) fish species. Significant changes are highlighted in **bold**

	Potamodromous species Iberian barbel (*L. bocagei*)			Resident species Iberian chub (*S. carolitertii*)		
Life-cycle trait	*NRR (River Vez)*	*RR (River Homem)*	*Response to flow regulation*	*NRR (River Vez)*	*RR (River Homem)*	*Response to flow regulation*
Longevity	Males: 6 years / Females: 10 years	Males: 7 years / Females: 10 years	↑ (Males only)	Males: 6 years / Females: 7 years	Males: 7 years / Females: 7 years	↑ (Males only)
Max. potential length (L_∞)	Males: 404 mm / Females: 674 mm	Males: 806 mm / Females: 875 mm	**↑ (Both sexes)**	Males: 184 mm / Females: 251 mm	Males: 170 mm / Females: 284 mm	= (Both sexes)
Growth rate (*K*)	Males: 0.11 / Females: 0.17	Males: 0.03 / Females: 0.06	**↓ (Both sexes)**	Males: 0.30 / Females: 0.35	Males: 0.34 / Females: 0.37	= (Both sexes)
Annual growth increments (average)	Males: 23.1 mm / Females: 33.2 mm	Males: 25.5 mm / Females: 30.8 mm	**↓ (Only females)**	Males: 13.2 mm / Females: 18.7 mm	Males: 13.1 mm / Females: 20.7 mm	= (Both sexes)
Seasonal growth pattern	1 period of significant growth (spring-summer)/1 period of no growth (winter)	Growth during all year but less significant	**≠ (Both sexes)**	1 period of significant growth (spring-summer)/1 period of no growth (winter)	1 period of significant growth (spring-summer)/1 period of no growth (winter)	= (Both sexes)
Growth type (allometric *vs* isometric)	Positive allometric	Isometric	**≠ (Both sexes)**	Isometric	Positive allometric	**≠ (Both sexes)**
Weight-length relationship (i.e., Body condition)	Low body condition	Low body condition	= (Both sexes)	Higher in smaller fish / Lower in larger fish	Lower in smaller fish / Higher in larger fish	**≠ (Both sexes)** **Ontogenic variability**
Age at first maturation	Males: 3^+ / Females: 5^+	Males: 3^+ / Females: 5^+	= (Both sexes)	Males: 2^+ / Females: 3^+	Males: 2^+ / Females: 3^+	= (Both sexes)
Gonad maturation pattern (*GSI*)	Longer maturation / Shorter spawning	Shorter maturation / Longer spawning	**≠ (Both sexes)**	Shorter spawning period (June)	Extended spawning period (May-June)	**≠ (Only females)**
Fecundity (min-max oocyte number/fish)	1429 - 6294 oocytes	1837 - 5882 oocytes	= (Females)	3628 - 5607 oocytes	2692 - 7275 oocytes	**↓ (Females)**
Oocyte size distribution	2 egg batches	1 egg batch	**≠ (Females)**	2 egg batches	1 egg batch	**≠ (Females)**

4.3. MANAGEMENT APPLICATIONS

The interface between water use and management by human populations and the maintenance of the integrity of aquatic ecosystems represents a difficult challenge to resource managers (Postel *et al*., 1996). An integral part of the strategy for meeting this challenge is to understand how past and present water management has affected the flow regime of river ecosystems and how the resulting flow regimes have affected aquatic biota (Brown & Bauer, 2010). Findings presented in this study corroborate the assumption that some life history traits of cyprinid fish species can be significantly altered downstream of dams, in the order of only a few decades and promote a high degree of intra and interspecific differences in response to flow regulation. This type of evaluation of the interplay between environmental variability, its artificialization, and bio-ecological cycles of different fish species is of increasing importance nowadays, specially within the joint contexts of increasing river fragmentation, flow regulation by dams and climate change (Xenopoulos *et al*., 2005). Therefore, findings from this study will be important to understand the derivation in biological patterns imposed by increasing river regulation on freshwater fish species and to be used as guiding elements for flow requirements implementations (Palmer *et al*., 2008; Arthington, 2012).

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