

Life history traits of the endangered Iberian loach *Cobitis calderoni* in the River Lozoya, Central Spain

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Abstract. Life history traits of the vulnerable Iberian loach *Cobitis calderoni* were studied in the River Lozoya, a regulated river in Central Spain. A total of 163 specimens were collected on five occasions, from April to December 1990. In general *C. calderoni* grew isometrically and a length-frequency analysis showed 4–5 age classes. Parameters fitted to the von Bertalanffy growth model showed variation in growth rates between the sexes. The gonadosomatic index for both sexes were similar and varied among months, with the highest values in April, just before the onset of the spawning season in Spring.

Key words: egg size, fecundity, growth, length frequency, von Bertalanffy growth function

Introduction

Cobitis calderoni Bacescu, 1962 which is one of the three endemic Iberian loaches, is distributed throughout the great basins of northern Spain; i.e. in the Duero, Ebro and northern areas of the Tajo river basins (Doadrio & Perdices 1997). Like other Iberian loaches, this species has been included in the Bern Convention (82/72, Annex III) and catalogued as vulnerable in the Red Book of Spanish Vertebrates (Doadrio 2001). Interest from ichthyologists has mostly concerned its distribution (Doadrio 1981, Alvarez et al. 1985, Docampo & Rallo 1987, Perdices & Doadrio 1997), and taxonomy and genetic variability (Madeira et al. 1992, Vasileva et al. 1992, Perdices et al. 1995, Perdices & Doadrio 2000, 2001). Despite these studies, its life history has been neglected; there are only two papers concerning the feeding ecology of *C. calderoni* (Valladolid 1996, Valladolid & Przybylski 2003), and little information about the species was provided when it was first described (Bacescu 1962). However, baseline data on the life history characteristics of fishes under threat are a prerequisite for conservation programmes (Wotton et al. 2000). Therefore the aim of this study was to provide detailed information on specific aspects of the biology of this species.

Study Area, Material and Methods

Specimens of *C. calderoni* were obtained from the River Lozoya (Tajo Basin, Central Spain), an example of a river highly modified by a series of dams and reservoirs. The sampling site was located in the lower part of the river between two reservoirs i.e. El Atazar (downstream) and La Parra (UTM 30T 461252 4528800). The flow regime at the site was regulated by the dam outflow, varying from a limited discharge (minimum flow under 0.5 m³ s⁻¹) to a

non-existent discharge for most of the year, when the river is reduced to a series of pools of varying depth and size. In 1990, isolated ponds along the river bed formed from July to September. Further information about the sampling site is available in Valladolid & Przybylski (1996).

Fish were collected using a DC electrofishing unit (220 V, 1.5 to 2.5 A) in April, May, July, September and December 1990. Specimens were anaesthetised, fixed in 10% formalin and preserved in 70% ethanol. In the laboratory, fish were measured for standard length (SL) and weighed to the nearest 0.1 cm and 0.01 g, respectively. Sex was identified by gonad examination under a binocular microscope.

Growth analysis was based on a length-frequency analysis, followed by the application of a modal class progression analysis. Size classes were presumed to represent annual age classes identified in seasonal length-frequency samples using the method of Bhattacharya (1967). Modal class progression analysis was conducted with a minimum class separation index of 2 (Gayaniolo et al. 1994). Results from length-frequency decomposition of the June sample were used to describe the growth pattern using the von Bertalanffy model (Ricker 1975):

$$L_t = L_{\infty} (1 - \exp(-k(t-t_0)))$$

where L_t is standard length (mm) at age t (years) and L_{∞} is the asymptotic length. Parameters of the von Bertalanffy growth function (VBGF) and their standard errors were estimated using a non-linear least-square regression with Marquardt's algorithm (Marquardt 1963) implemented with FiSAT (Gayaniolo et al. 1994), following same methodology applied to similar data for *C. paludica* (Przybylski & Valladolid 2000). Because the reversed relationship between L_{∞} and K is an inherent property of the VBGF (Moreau et al. 1985), to compare the growth pattern between males and females, the index of growth performance ($\phi' = \log_{10}[k] + 2 \times \log_{10}[L_{\infty}]$) (Munro & Pauly 1983) was used.

To assess the presence of an allometric growth pattern (Ricker 1975), the weight-length relationship was determined by linear regression. Differences between the sexes in the form of the relationship were tested with ANCOVA, and for isometric growth with a t-test (Quinn & Keough 2002).

Reproductive parameters (fecundity, oocyte diameter, gonadosomatic index) were estimated on the basis of gonad analysis. Gonads were removed, weighed to the nearest 0.0001 g and the number of eggs counted. The diameters of 30 randomly selected oocytes from each female were measured and pooled data were used to obtain a size-frequency histogram. Fecundity was expressed as total number of oocytes found in gonads before the spawning season, in this case, in April. The gonadosomatic index (GSI) relating gonad weight to total body weight, as a measure of reproductive effort, was calculated for each mature male and female.

Results and Discussion

A total of 163 specimens of *C. calderoni* were caught. The sample comprised 92 females and 68 males, with 3 specimens not sexed due to their small size (24–30 mm SL). The overall sex ratio was 1.5:1 (F:M) and did not differ significantly from unity ($\chi^2=2.977$, $p=0.0845$). Baccescu (1962) highlighted a pronounced female bias in the sex ratio, like that noted for some populations of *C. paludica* (Przybylski & Valladolid 2000, Soriguer et al. 2000, Oliva-Paterna et al. 2002). In the genus *Cobitis* such female biased sex ratios is quite common (Bohlen & Ritterbush 2000) and several mechanisms may

explain the observation. The scarcity of one sex in a population could arise through a sex bias in sampling, which is possible for bottom dwelling fishes (Zalewski & Cox 1990), or to size (and therefore sex) related habitat preferences (Baccucci 1962).

The length-frequency distribution of *C. calderoni* in the Lozoya revealed large differences between males and females (Fig. 1). Maximum female standard length was 62 mm (a specimen caught in April) which was 14 mm larger than the biggest males; i.e. 48 mm SL. A similar situation was found in *C. paludica* (Przybylski & Valladolid 2000) and in other *Cobitis* species; e.g. *C. elongatoides* (Eros 2000), *C. narentana* (Schneider et al. 2000, Zanella et al. 2003), and *C. taenia* (Slavík & Ráb 1995, Kostrzewa et al. 2003).

Separate length-frequency distributions for males and females did not show clear cohorts (Fig. 1a). Therefore the Bhattacharya method followed by modal class progression analysis was used for pooled data. Only in a sample collected in July were several Gaussian components clearly necessary to explain the length-frequency distribution (Fig. 1). For this month the most likely solution consisted of 4 groups with a separation index values greater than 2 (Table 1). The explained distribution was not significantly different from the observed ($\chi^2=11.19$, $df=8$, $p=0.1912$) though tests with $df < 10$ are often unreliable (Gyani lo

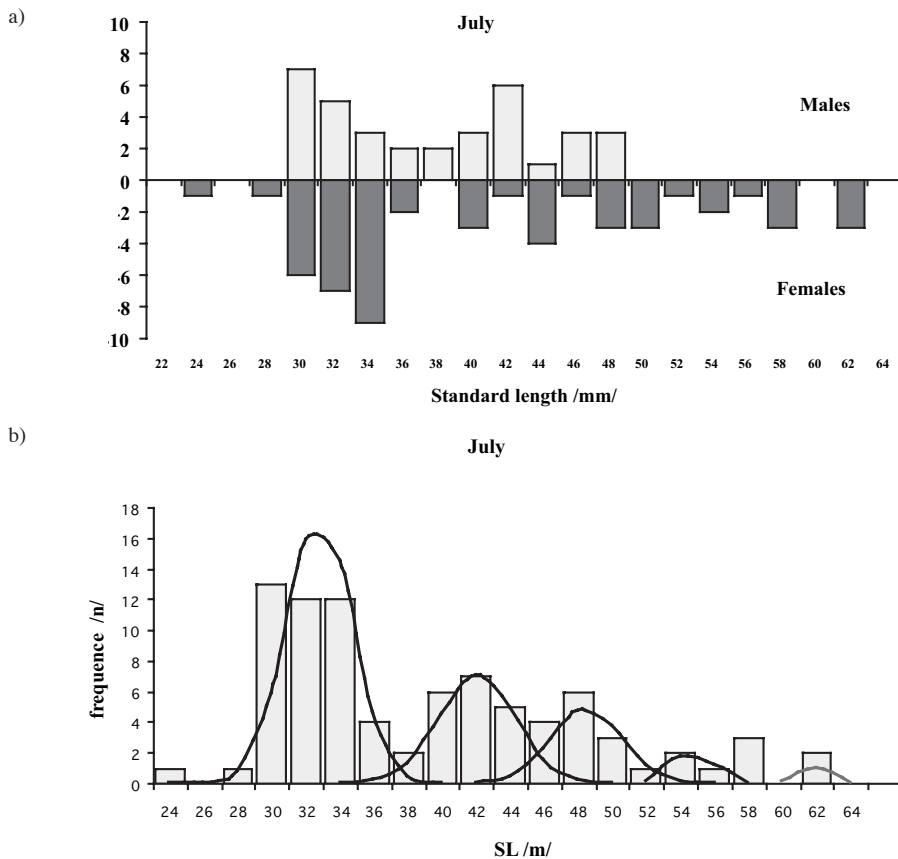


Fig. 1. Length-frequency diagram of *Cobitis calderoni* from the River Lozoya. (a) Standard length differences between male and females, (b) Bhattacharya decomposition of length frequency into groups presumed to represent age classes.

et al. 1994). Considering that the Bhattacharya method is not able to produce a normal distribution for the last mode (Fig. 1b), the number of separated groups could be increased to 5. These separated groups are likely to represent age classes, if so, the lifespan of males is shorter than females. This pattern was also found in a previous study of *C. paludica* at the same location (Przybylski & Valladolid 2000) as well as in other south-west European populations (Oliva-Paterna et al. 2002).

Fitting the length-at-age data, obtained from a decomposition of the length frequency diagram to the von Bertalanffy model, variation in growth rates between sexes is evident (Table 2). High values of the coefficient of determination show that this function fits all the data well, though the asymptotic length appears to be overestimated according to Taylor's criterion (Taylor 1962). The growth rate of *C. calderoni* was of the same order of magnitude as for *C. paludica* (Przybylski & Valladolid 2000, Soriguer et al. 2000, Oliva-Paterna et al. 2002); females were larger than males and the asymptotic length (L_{inf}) was almost 1.5 times higher for females than males. Further differences in growth parameters between males and females are revealed by values for the performance growth index (Table 2).

Contrary to the sex differences in life histories mentioned above, the weight-length relationship calculated separately for males and females (for each sampling occasion) showed no differences in the slopes of the regressions between the sexes, though there were differences between samples (Table 3). Moreover, the result showed that *C. calderoni* largely grows isometrically but with deviations from $b=3$ in April and July (Table 3).

The gonadosomatic index differed throughout the year (Fig. 2), but there were no significant difference between males and females from May to December (ANOVA $F=72.509$; $df=9,114$; $p<0.001$). Only female GSI in April was higher than the male GSI (post hoc Tukey test). For this month GSI was significantly related to female size (log GSI =

Table 1. Decomposition of length-frequency data of *C. calderoni* standard length (S.L. = standard length, S.D.= standard deviation, n= number of specimens) of the River Lozoya population (sampled in July) using the Bhattacharya method followed by the modal class progression analysis. The value of the separation index (S.I.) must be greater than 2 for meaningful separation of the groups.

Group	n	S.L. (mm)		S.I.	estimated age
		average	SD		
1	42	32.6	1.91		1+
2	20	41.9	2.24	4.464	2+
3	13	48.3	2.04	3.013	3+
4	6	57.8	2.41	4.239	4+

Table 2. Estimation of von Bertalanffy parameters and their asymptotic standard errors (in parentheses) for *C. calderoni* population in the River Lozoya.

	Females		Males	
L_{inf}	91.5	(18.26)	61.5	(19.16)
K	0.186	(0.073)	0.224	(0.163)
t_0	-1.164	(0.335)	-1.76	(0.841)
ϕ'	3.192	(0.287)	2.928	(0.495)
n	105		34	
r^2	0.9317		0.95431	

Table 3. Regression of body weight on standard length (log - transformed data) for *C. calderoni* from the River Lozoya. Asterisks show differences in growth patterns (i. e. allometric if $b \neq 3$ or isometric $b = 3$). There was a lack of differences in slope (coefficient b) between sexes in each month.

Month	a	s.e. a	b	s.e. b	r ²	n	p
April *	-4.231	0.184	2.420*	0.107	0.983	11	<0.001
May	-6.050	0.715	3.477	0.447	0.924	7	<0.001
July *	-5.012	0.082	2.799*	0.052	0.972	68	<0.001
September	-4.826	0.134	2.692	0.083	0.966	39	<0.001
December	-5.386	0.227	3.074	0.138	0.965	20	<0.001

$$F_{5,135} = 2.7826 \quad p = 0,020$$

Multiple comparisons (Tukey test)

M D J S A

* slope is significantly different from $b = 3$

5. $477 \times \log SL - 8.282$; $r^2=0.969$). In general, during the reproductive season the variation in GSI had a range comparable to that observed by Lobon-Cervia & Zabalá (1984), Halačka et al. (2000), Erős (2003) and Kostrzewa et al. (2003).

Similarly like for other *Cobitis* species (Marconato & Rasotto 1989, Halačka et al. 2000, Erős 2003, Juchno & Boroń 2006), the frequency distribution of egg diameters (Fig. 3) was multimodal, encompassing oocytes at different stages of development. Such asynchronous oocyte development classifies this fish as a multispawning species. The largest oocytes (from 1.2 to 1.8 mm in diameter) at the vitellogenesis stage were observed only in April and May and a similar size of oocytes was recorded by Bacescu (1962). Small size previtellogenic oocytes dominated through the year. In this situation the measurement of fecundity is more complicated (Halačka et al. 2000) but, assuming that all oocytes could mature during the spawning season, total fecundity could be measured as the number of all oocytes in the ovaries. Using this assumption, fecundity was related to female SL by the equation: $Ft = 0.0035 \times SL^{3.0362}$, $r^2=0.667$. Oocytes in the previtellogenic phase of development can be a reservoir of

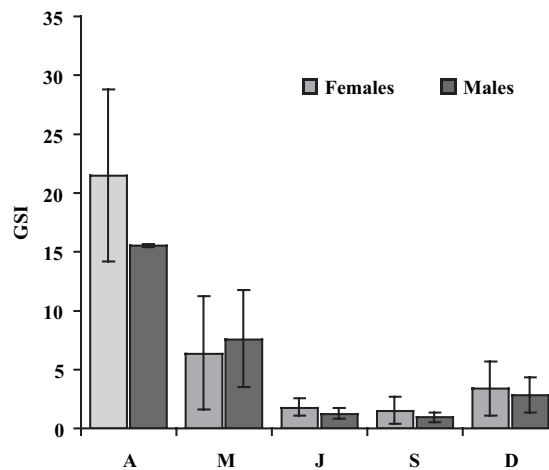


Fig. 2. Variation in gonadosomatic index of *C. calderoni*.

reproductive cells for subsequent years. Taking into account the number of mature eggs in the gonads (batch fecundity F_b), this relationship was not significant ($F_b = 0.00036 \times SL^{2.252}$, $r^2=0.255$, $p=0.247$). Because these data are based on the analysis of a small number of females ($n=9$) these results should be treated with caution.

In general *C. calderoni* was found to exhibit a life history pattern similar to that of *C. paludica* (Przybylski & Valladolid 2000, Oliva-Paterna et al. 2002). Both species utilise similar food resources (Valladolid & Przybylski 2003), and in both species males are smaller than females and have a shorter life span (3+ and 4+/5+, respectively). Both species grow isometrically and males achieve a similar

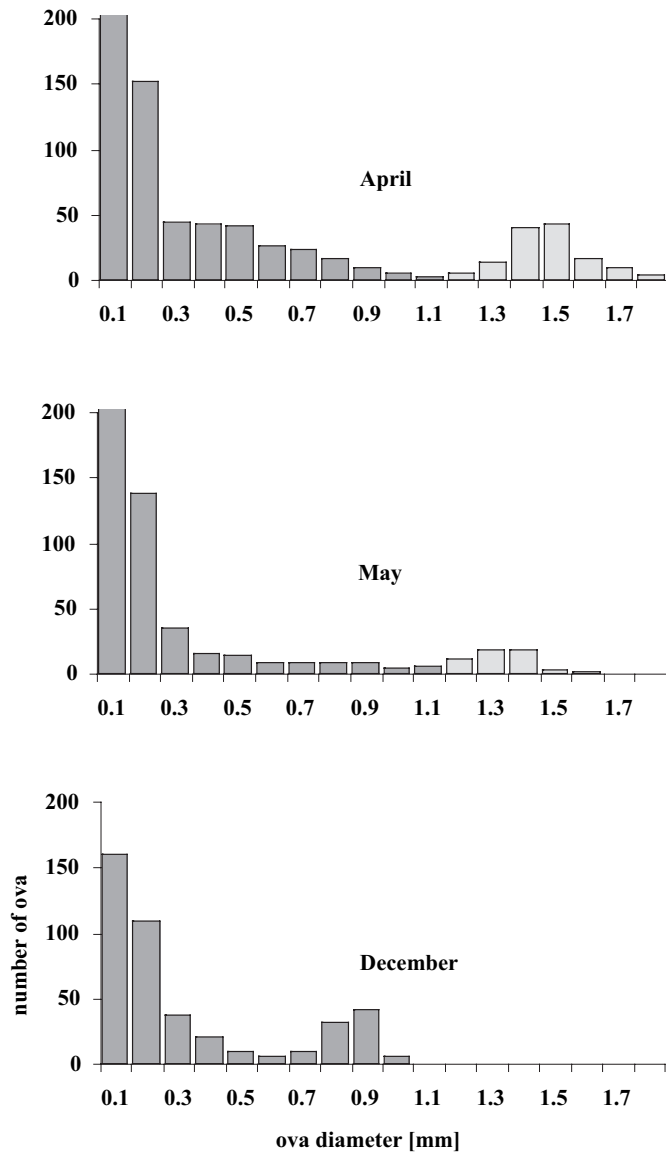


Fig. 3. Size-frequency distribution of oocytes from *C. calderoni* females.

lower asymptotic length than females (L_{inf} less than 100 mm for females). *C. calderoni*, like *C. paludica*, is a multispawner (Wotton 1990) and such a strategy can be seen as a trade off between traits that increase female fitness; i.e. simultaneous increase in the number and size of eggs. However, both species can differ in time of spawning. The maximum GSI for *C. paludica* was observed in May (Przybylski & Valladolid 2000) but in April for *C. calderoni*. These similarities in life history traits may result from the typical ‘cobitoid’ pattern of life history, responding in similar ways to selection in the relatively harsh environmental conditions of the seasonal Iberian stream environment (Gasith & Resh 1999).

Many fish species, among them the stone loach (*Barbatula barbatula*), display a geographical variation in life history characteristics (Mann et al. 1984). Although this aspect of life history evolution has not been explored for *Cobitis* species, further differences in life history traits between Iberian loaches may occur between northern populations of *C. calderoni* and southern ones of *C. paludica*.

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