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Biology of the endangered cyprinodontid *Aphanius iberus* in a saline wetland (SE Iberian Peninsula)

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Abstract

For the last few decades, *Aphanius iberus*, an endemic cyprinodontid from the Iberian Peninsula and included in international red lists, has been declining despite several management efforts. In this study we present the biology of the species in an isolated wetland managed for salt exploitation (its most common habitat type in the southeastern Iberian Peninsula). The population studied is found under conditions of extreme high salinity and water temperature. We have examined the effects of extractive management on catchability, population structure, growth and reproduction traits. The stock was characterized by significant differences in abundance between seasons (higher values: end of summer and early autumn). Drastic changes in salinity and water level have negatively affected the species abundance. The population structure was characterized by three age groups (0+, 1+ and 2+ in both sexes), a high degree of group-size overlapping into the 0+ (>2 groups), a short life span and a long reproductive period (April/May to September). Gonadal mass represented a maximum of 19.6 ± 0.5% of the total mass in females. We detected changes in life-history traits which could be related to the management of the salt extraction, variations in water level that increase the salinity should be indirect effects on fish population by reducing vegetation mats.

Keywords: Aphanius iberus, *age, population structure, somatic condition, reproduction, management effects*

Introduction

The Spanish toothcarp *Aphanius iberus* (Valenciennes, 1846) is an endemic cyprinodontid of the Iberian Peninsula restricted to a few populations along the Spanish Mediterranean coastline (Oliva-Paterna et al. 2006a). The species is catalogued as endangered (EN) and is one of the few Iberian fish species protected by national and international laws (Elvira 1995; IUCN 2006).

Under the need to increase the knowledge of life-history characteristics of threatened species as a necessary tool for their conservation, several papers on various life history and biological characteristics of *Aphanius iberus* have recently been published including genetic variability (Araguas et al. 2007), feeding ecology (Alcaraz & García-Berthou 2006; Alcaraz et al. 2008), ecophysiology (Oliva-Paterna et al. 2006b) and interactions with invasive species (Rincón et al. 2002; Caiola & De Sostoa 2005). However, its growth pattern and reproduction have only been studied in two populations located in the most northern area of its distribution range (García-Berthou & Moreno-Amich 1992; Vargas & De Sostoa 1997).

As with other native Mediterranean cyprinodontids, *Aphanius iberus* current distribution has mostly been reduced to saline waters (Alcaraz et al. 2008), mainly eusaline and hypersaline. Moreover, the importance of salt exploitation wetlands as the most typical habitat of the species in the south-eastern of the Iberian Peninsula has been noted (Oliva-Paterna et al. 2006a). However, the population dynamic in this extreme environment is unknown.

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This study presents the first data on the life-history traits of a population of *Aphanius iberus* at the most southern area of its distribution range and located in a managed hypersaline habitat with extreme water temperatures all the year. The objectives of our paper are (i) to provide information about the biology and to test whether the population dynamic differs in size structure, growth and reproduction parameters from other populations and (ii) to test for possible effects of the management on life-history characteristics. We hypothesized that *Aphanius iberus* might use this managed wetland, displaying different features from more natural habitats, which would have important management implications for the conservation of this and similar endangered wetland fishes.

Materials and methods

Study area and sampling methods

The population studied inhabits a small isolated wetland (Marchamalo; UTM 30SYG06) managed for salt exploitation in the south part of the Mar Menor, a large coastal lagoon located in the SE of Spain (Pérez-Ruzafa et al. 2005). The study was carried out into two contiguous small pools (2 ha) characterized by low depth (<50 cm), soft substrates (muddy bottoms) and isolated patches of sumerged vegetation and mats (*Ruppia* spp. and *Cladophora* spp.). The effects of inmigration into the studied population are minimal because of salt extraction management which did not take biological criteria into account, the water entrance channel of the pools was periodically blocked, and outmigration is absent because the wetland has no natural communication with the Mar Menor lagoon. In fact, the temporal connections with the coastal lagoon is only occasional; during the study period, a water entrance was realised through hydraulic pumps no more than four times per year.

From May 2000 to June 2002, a total of 6007 individuals of *Aphanius iberus* was captured on 28 sampling days (μ_1–μ_{28}) in the two pools with similar density and homogeneous size groups of fish. Each stratified sample consisted of a combined process of sieving (quadrangular hand nets 40 × 40 cm; 1 mm mesh size) for 15–20 min and setting 10–20 minnow traps (30 mm Ø; 1 mm mesh size) (Harrison et al. 1986) uniformly distributed in each pool for roughly 24 h.

In the field, only a random sample of 2612 individuals anesthetized with benzocaine were sexed (male, female or undifferentiated) and measured (total length, L_T, ±1 mm). A total of 997 individuals (about 40 individuals per sample; 16.6% of the total captures) was preserved in neutralized formaldehyde (10%), 1615 individuals were released unharmed. In the laboratory, besides sex and length, eviscerated mass (M_E, ±0.01 g) and gonadal mass (M_G, ±0.001 g) were registered with a precision balance (0.0001 g), within 15 days from its capture.

Age structure was assessed using both scales and monthly and seasonally length–frequency distributions. Several scales (6–10) between the dorsal fin and lateral line were removed, cleaned (8% NaOH) and mounted dry between two slides for stereomicroscope study from 153 females and 150 males.

Water level, temperature, salinity, and submerged vegetation were registered for each sampling period. Water temperature showed an annual pattern of variation, with maxima (31.6°C) in summer (July–September) and minima (11.3°C) in winter (December–February) (Figure 1). Average salinity was 47.27 and remained quite stable during the studied period, depending mainly on the quantity of sea water entering the pools, so silting of the entrance channel produced atypical increases in salinity, reaching maxima of 71.00 (August 2000) and 83.67 (April 2001) and minima of 28.00 (January 2001) and 39.00 (November 2001) (Figure 1).

Water level fluctuation was categorized as the percentage of the relationship between minimum and maximum water volume (four categories: 25, 50, 75 and 100%). Maxima levels coincided with rainy periods and minima with management for salt extraction (Figure 2). Submerged vegetation was evaluated as the percentage of 6 sampling units of 50 m^2 located in the shore line and classified as absent (non-aquatic vegetation), little (covering less than 10%), poor (covering 10–30%), disturbed (covering 30–60%), and developed (covering more than 60% of the sampling unit). Although the submerged vegetation recovered slightly in both spring and summer periods, the overall trend during the study period was to decrease (Figure 2).

Statistical analysis

The total number of individuals caught by minnow-traps was counted and expressed as catches per unit of effort (CPUE), 1 unit being a passive trap in place for 12 h, log-transformation was carried out.

Monthly, seasonal and size differences in sex ratio were analysed using chi-squared tests. Age validation was accomplished using a length–frequency-based method available in the FiSAT II software (FAO ICLARM Stock Assessment Tools Ver.1.2.0). Size groups presumed to represent cohorts or age classes in the length–frequency seasonal samples were

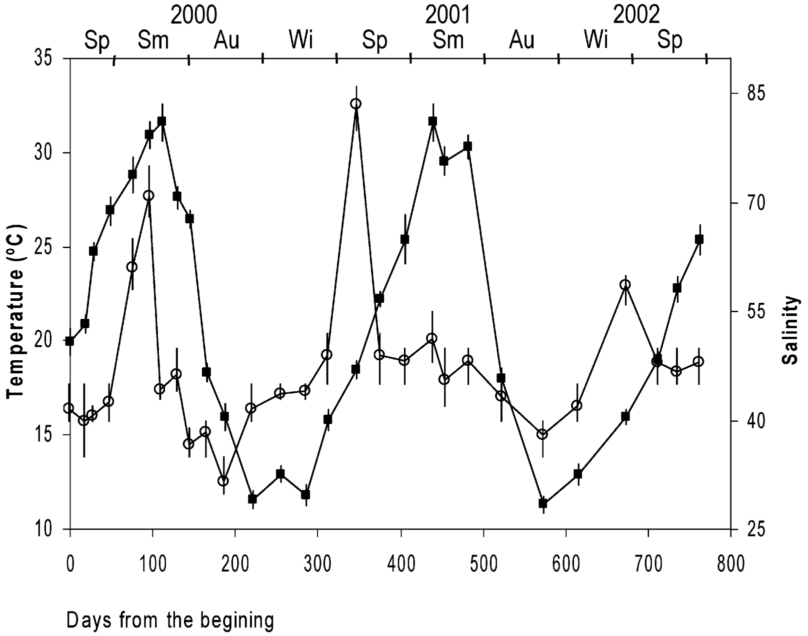

Figure 1. Seasonal changes in water temperature (°C; black squares) and salinity (white circles) of the Marchamalo wetland. Mean ± 95% CL of values for six of more surface samples. Marks separate the seasons: (Sp) spring, (Sm) summer, (Au) autumn and (Wi) winter.

identified by the method described by Bhattacharya (1967). This was followed by the separation of the normally distributed components (NORMSEP subroutine); the value of the separation index (SI) must be >2 to provide meaningfully separated groups (Gayanilo et al. 1988).

Temporal variation in somatic condition and gonadal development was studied using analysis of

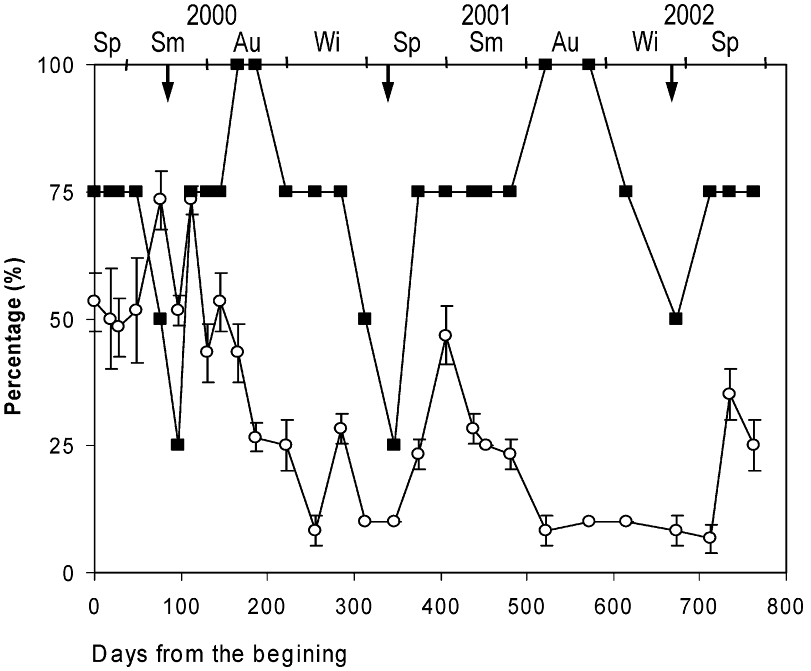

Figure 2. Seasonal changes in water level fluctuation (percentage of the relationship between minimum and maximum water volume; black squares) and submerged vegetation (percentage in sampling units; white circles) of the Marchamalo wetland. Mean of values for six of more samples. Marks separate the seasons: (Sp) spring, (Sm) summer, (Au) autumn and (Wi) winter.

the length–weight relationships. The adjustment for size variation in the data by regression-related techniques has been used with ANCOVA as a successful method for *Aphanius iberus* (García-Berthou & Moreno-Amich 1993; Vila-Gispert & Moreno-Amich 2001). This method applies analysis of covariance (ANCOVA) using M_E as the dependent variable and L_T as the covariate. A log-transformation is made to obtain a linear relationship between M_E and L_T. The homogeneity of the regression coefficients (slopes) is tested with an ANCOVA that analyzes the pooled covariate-by-factor interaction. If the covariate-by-factor interaction (homogeneity of slopes) is not significant ($P>0.05$), standard ANCOVA would be applied to obtain predicted values that are fitted according to L_T (predicted M_E values). Seasonal variations in somatic condition were studied by using the predicted M_E values, testing differences with ANOVA tests (Tukey's HSD post hoc tests). Gonadal development was studied using the same ANCOVA method, with M_G as the dependent variable and L_T as the covariate. Predicted M_G values were analyzed temporally (ANOVA tests; Tukey's HSD post hoc tests).

We also analyzed bivariate relationships between environmental variables, species relative abundance (CPUEs), predicted M_E values and predicted M_G values using Spearman's correlation coefficients (R_S).

Results

Abundance and sex ratio

The temporal pattern of minnow trap CPUE estimates for *Aphanius iberus* showed a high degree of monthly and seasonal variation (ANOVA, season as a factor: $F_{(8,426)}=44.545$, $P<0.001$) (Figure 3). Maximum CPUE means appeared at the beginning of the study ($\text{CPUE}_{\text{Sp2000}}=40.40\pm7.37$) and progressively decreased throughout the study period. CPUEs were significantly lower for the second year of the study (Cycle-2: spring 2001–spring 2002 inclusive) ($\text{CPUE}_{\text{Cycle-1}}=17.38\pm2.67$; $\text{CPUE}_{\text{Cycle-2}}=9.90\pm1.21$; Student's $t=3.66$, $P<0.001$). We detected minima values in winter periods ($\text{CPUE}_{\text{Wi2000–01}}=4.24\pm1.33$; $\text{CPUE}_{\text{Wi2001–02}}=4.25\pm1.75$), although a non-increase period of abundance was also seen in spring 2001 ($\text{CPUE}_{\text{Sp2001}}=4.10\pm1.10$; Figure 3). Furthemore, significant and drastic drops of CPUE values were observed in samples at the beginning of summer 2000 (μ_5, μ_6, μ_7 in Figure 3) and in the sample of 17 April 2001 (μ_{16} in Figure 3).

During the study period, bivariate relationships were detected between three environmental variables (water temperature, salinity and submerged vegetation) and species relative abundance (log CPUE+1) (Table I).

There was a significant difference from 1:1 in the overall sex ratio (1005 males to 1294 females, 0.79:1; $X^2=35.13$, $P<0.001$). The number of males was significantly higher (1.45:1; $X^2=38.15$, $P<0.001$) in the lower length classes ($L_T<30$ mm). Females were significantly dominant during spring 2000 ($X^2=6.69$, $P=0.009$), summer 2000 ($X^2=133.1$, $P<0.001$), autumn 2000 ($X^2=12.92$, $P<0.001$) and spring 2002 ($X^2=6.39$, $P<0.011$). In spring and summer 2001 sex ratio did not differ significantly from 1:1 ($P>0.05$). The rest of the seasons was significantly dominated by males (winter 2000–01, $X^2=4.58$, $P=0.032$; autumn 2001 $X^2=9.60$, $P=0.002$; winter 2001–02 $X^2=95.2$, $P<0.001$).

Age and population structure

Three age groups (0+ to 2+ years) were detected in both sexes. The maximum lengths observed were a 60 mm female caught in February (2001) and a 45 mm male caught in June (2002). The catchability of specimens with length less than 10 mm was practically null; however, its occurrence was confirmed during the recruitment periods. Females ($L_T=32.7\pm0.4$ mm) were significantly (ANOVA, $F_{(1,2287)}=366.06$, $P<0.05$) longer than males ($L_T=28.1\pm0.3$ mm). A sex-related difference between sizes was statistically defined for all seasons except summer 2001 (ANOVA, $F_{(1,142)}=2.37$, $P=0.126$; females, $L_T=24.3\pm1.1$ mm; males, $L_T=23.2\pm0.9$mm).

The Bhattacharya method followed by the modal class temporal progression analysis using the length–frequency of the seasonal samples identified probable modal distributions, with significant separation indices (SI>2) of three cohorts (=size groups) in males (Table III) and four cohorts in females (Table II). These were detected in all seasons except autumn 2000 for males (Table III) and summer 2001 for females. Age determination by scales allowed to assign an age to each cohort (Tables II and III).

Using both methods, the population structure was characterized by three age groups (0+ 77.51%; 1+ 21.54% and 2+ 0.95%) with a minimum of two consecutive cohorts included in the 0+ age group for males and three for females in each season. The maximum percentage of specimens with *annulus* appeared in spring (mainly April and May).

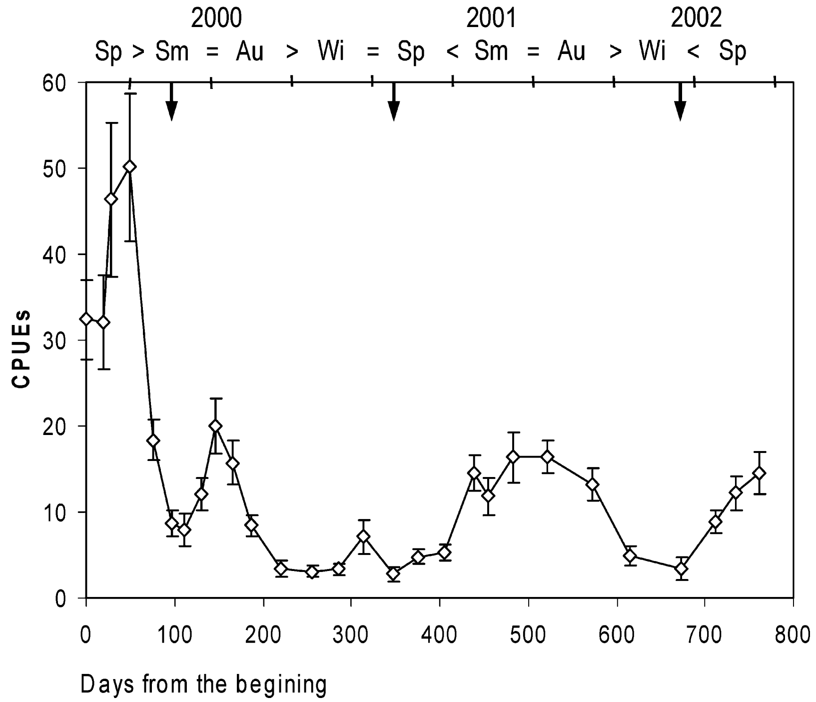

Figure 3. Seasonal changes in relative abundance for total individuals of *Aphanius iberus*. Mean ± 95% CL of CPUE values for samples of 25 or more fish (CPUE=Catch per unit of effort). Results of Tukey's HSD tests ($P \leq 0.05$) separate the seasons: first Sp=CPUE mean of Spring 2000, etc.; black arrows mark maxima in water salinity.

Modal progression analysis of length frequency distributions along the study period showed a continuous presence of individuals shorter than 20 mm L_T, and a very long recruitment period from June to early November (cycle-1) and from May to October/November (cycle-2). Winter samples showed the presence of individuals from 12 to 44 mm L_T (Figure 4a). In contrast, in August 2000 and April 2001 no immature individuals were captured, even though these months fell within the reproductive period (Figure 4b). These samples coincided with peaks in salinity and massive fish mortality was detected during the first.

Somatic condition and gonadal development

The results of the ANCOVAs to estimate the effects of the factors (sex and season) on the L_T–M_E and L_T–M_G relationships are shown in Table IV. In the preliminary design (test for interaction) carried out with both males and females a significant interaction between covariate (L_T) and season was found. This indicates temporal heterogeneity in the slopes of the relationships, which was also observed in the ANCOVAs by sexes separated.

Significant changes were obtained in the temporal variation of the predicted M_E values (=Somatic condition index) for males and females (Table V and

Table I. Correlation matrix of relative abundance (CPUEs), predicted M_E values (condition index), predicted M_G values (gonadosomatic index) and environmental variables for all samples in the population studied (Spearman's correlation significant coefficients*).

		Predicted M_E values		Predicted M_G values	
Environmental variables	CPUEs	Males	Females	Males	Females
Water temperature	0.43*	0.37*	0.34*	0.72*	0.73*
	P=0.023	P=0.055	P=0.072	P<0.001	P<0.001
Salinity	−0.35*	−0.09	−0.12	0.24	0.34*
	P=0.069	P>0.1	P>0.1	P>0.1	P=0.079
Water level fluctuation	0.19	0.03	−0.06	0.10	0.21
	P>0.1	P>0.1	P>0.1	P>0.1	P>0.1
Submerged vegetation	0.48*	0.56*	0.47*	0.52*	0.59*
	P=0.011	P=0.002	P=0.011	P=0.004	P=0.001

Table II. Length frequency data (L_T; mm) of females of the Marchamalo wetland using the method of Bhattacharya (1967) followed by the separation of the normally distributed components (Gayanilo et al. 1988), the separation index (SI) must be greater than 2 to provide meaningfully separated groups. Estimated age groups are confirmed by scale analysis.

		L_T (mm)				
Samples	Groups	average	SD	*n*	SI	Estimated age
Spring 2000	1	14.71	1.98	7	–	0+
	2	32.35	3.01	111	7.08	0+
	3	38.04	2.54	124	2.05	0+
	4	46.54	1.27	10	4.48	1+
Summer 2000	1	14.10	2.02	4	–	0+
	2	31.18	4.59	437	5.17	0+
	3	39.16	1.67	97	2.55	1+
	4	47.44	1.24	46	5.67	1+/2+
Autumn 2000	1	15.94	2.41	22	–	0+
	2	22.30	1.28	25	3.44	0+
	3	29.66	3.25	155	3.25	0+
	4	38.26	1.68	12	3.50	1+
Winter 2000–01	1	14.38	2.05	38	–	0+
	2	24.24	4.82	53	2.87	0+
	3	36.73	3.18	17	3.12	0+/1+
	4	60.00*		1		2+
Spring 2001	1	14.41	2.21	51	–	0+
	2	26.44	3.64	17	4.11	0+
	3	34.60	2.49	18	2.66	0+
	4	44.01	1.00	2	5.40	1+
Summer 2001	1	14.08	2.16	124	–	0+
	2	21.62	4.60	83	2.23	0+
	3	37.13	1.79	5	4.85	1+
Autumn 2001	1	16.33	1.08	27	–	0+
	2	20.19	1.14	5	3.46	0+
	3	27.05	2.80	10	3.47	0+
	4	38.00	1.00	2	5.75	1+
Winter 2001–02	1	16.35	1.86	27	–	0+
	2	23.62	2.70	17	3.19	0+
	3	31.33	1.11	16	4.04	0+
	4	36.94	1.49	8	4.32	1+
Spring 2002	1	17.00	1.64	3		0+
	2	23.00	1.00	1	4.54	0+
	3	33.31	2.88	9	5.31	0+/1+
	4	40.75	2.24	22	2.90	1+

* This individual was not introduced in the length–frequency distribution modal analysis.

Figure 5). A similar somatic condition cycle was evident in both sexes (Spearman's correlation of mean values: $R_s = 0.901$, $P < 0.001$), although differentiation of the repetitive phases was difficult. Minimum predicted M_E means appeared at the end of summer and in autumn. These increased in winter, but recovered to reach a maximum in spring, especially in the case of females. In addition drastic and significant drops in predicted M_E values were observed in some samples at the beginning of summer 2000 (μ_5, μ_6 in Figure 5) and the sample of 17 April 2001 (μ_{16} in Figure 5).

In both sexes, somatic condition was significantly lower during the second year of the study (May 2001–May 2002) (Males cycle-1, predicted $M_E = 1.35 \pm 0.22$; Males cycle-2, predicted $M_E = 1.16 \pm 0.24$; Student's $t = -8.22$, $P < 0.001$) (Females cycle-1, predicted $M_E = 1.57 \pm 0.31$; Females cycle-2, predicted $M_E = 1.32 \pm 0.32$; Student's $t = 8.79$, $P < 0.001$).

Table III. Length–frequency data (L_T; mm) of males of the Marchamalo wetland using the method of Bhattacharya (1967) followed by the separation of the normally distributed components (Gayanilo et al. 1988), the separation index (SI) must be grater than 2 to provide meaningfully separated groups. Estimated age groups are confirmed by scale analysis.

		L_T (mm)				
Samples	Groups	average	SD	*n*	SI	Estimated age
Spring 2000	1	14.71	1.98	7	–	0+
	2	26.69	1.71	86	6.50	0+
	3	31.16	2.54	105	2.10	1+
Summer 2000	1	15.13	2.73	11	–	0+
	2	23.92	1.34	42	4.12	0+
	3	30.14	3.04	201	3.03	0+/1+
Autumn 2000	1	16.58	2.80	26	–	0+
	2	25.22	2.11	81	3.51	0+
	3	30.16	3.25	42	1.84	1+
Winter 2000–01	1	14.58	2.14	43	–	0+
	2	24.66	3.17	88	3.79	0+
	3	35.26	1.62	5	4.42	1+
Spring 2001	1	14.32	2.11	50	–	0+
	2	22.98	1.48	25	4.82	0+
	3	29.45	2.27	16	3.46	1+
Summer 2001	1	13.73	1.96	115	–	0+
	2	19.60	2.96	76	2.39	0+
	3	28.70	1.57	14	4.01	1+
Autumn 2001	1	16.27	1.08	25	–	0+
	2	19.56	1.12	21	2.89	0+
	3	25.21	2.71	22	3.00	1+
Winter 2001–02	1	16.06	1.64	25	–	0+
	2	22.12	1.09	9	4.43	0+
	3	29.17	2.75	13	3.67	1+
Spring 2002	1	24.45	3.93	58	–	0+
	2	32.76	3.10	115	2.36	0+/1+
	3	45.00	1.00	1	5.97	2+

Temporal variation in gonad activity is shown as significant changes of the predicted M_G values (=Gonadosomatic Index) in Figure 6 and Table V. In both sexes (Spearman's correlation of mean values: $R_s=0.848$, $P<0.001$), three generic phases were identified in the annual cycle of gonad activity: quiescence (with minimum values of predicted M_G values), maturation and reproduction phase (when the predicted M_G values were greatest).

Quiescence occurred from the beginning of autumn (September–October) to the beginning of winter (January), after which gonad activity was reactivated. Thus, the population showed a quiescent period of three months, although this was less stable in the males of cycle-1 (Table V and Figure 6). In both sexes, gonad maturation (increase in predicted M_G values) occurred between the beginning of winter (January) and mid-spring (April–May). However, coinciding with an atypical increase in salinity, this phase showed no increase in female gonad weight and a decrease in gonad weight for males during cycle-1 (μ_{15}, μ_{16} in Figure 6).

Both sexes showed maximum predicted M_G values from mid-spring to mid-summer with the maximum values in June (μ_3, μ_{18}, μ_{28} males; μ_4, μ_{28} females; Figure 6), except females in August 2001 (μ_{21} females; Figure 6). These values represented a maximum of 19.6 ± 0.5% and 4.1 ± 0.7% of the total mass for ovaries and testes, respectively. The index decreased sharply between August (end of summer) and the begining of the quiescent period (Figure 6).

The pattern of gonad activity was similar in both study periods (Figure 6). In both sexes, however, the predicted M_G values of the second reproductive period were significantly lower (period 2000: μ_3–μ_6; period 2001: μ_{17}–μ_{21}) (period 2000: males predicted $M_G=2.12\pm0.03$, females predicted $M_G=2.97\pm0.05$; period 2001: males predicted $M_G=1.93\pm0.04$,

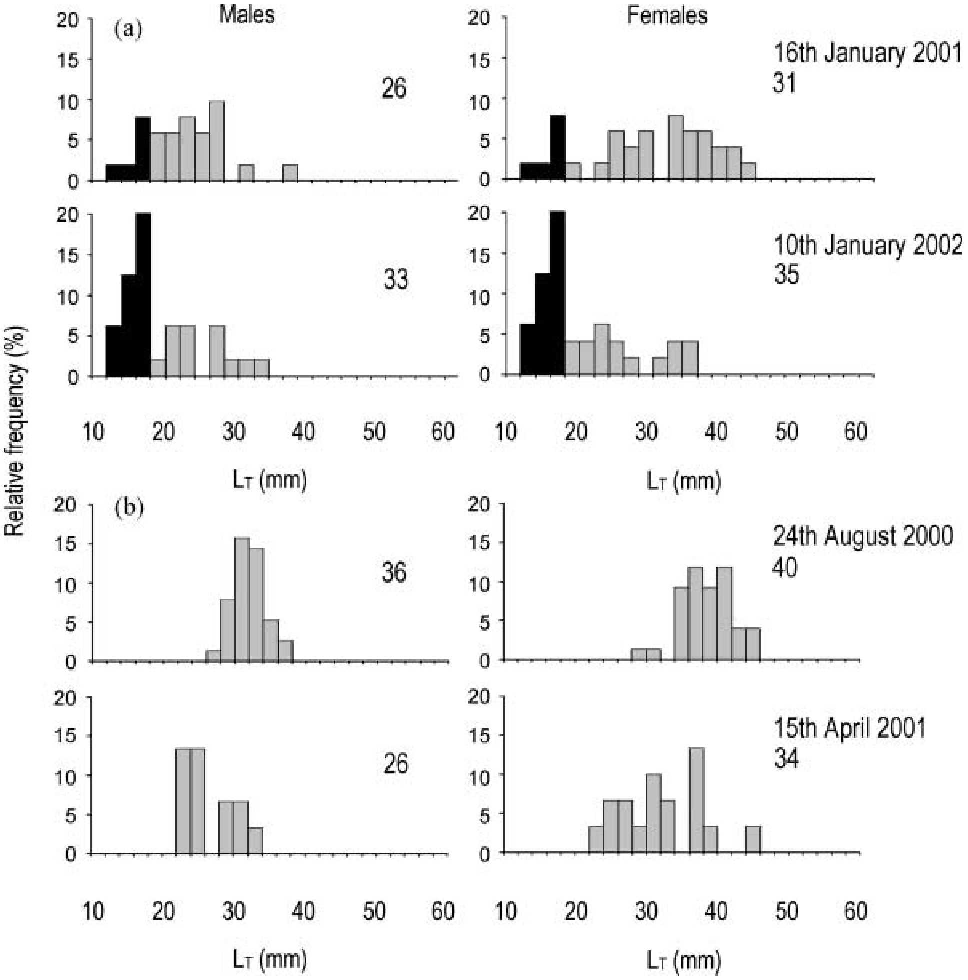

Figure 4. Length–frequency distribution of *Aphanius iberus* caught in sampling days included into winter seasons (a) and the reproductive period (b). Sexes and immature (black histograms) were separated. Numbers of individuals per sample and specific sampling dates are indicated.

females predicted $M_G=2.52\pm0.09$; males Student's $t=8.65$, $P<0.001$; females Student's $t=8.28$, $P<0.001$).

The correlation between water temperature and somatic condition showed marginally significant values ($P<0.1$; Table I), whereas there was a high degree of correlation between this environmental factor and gonad activity ($P<0.05$; Table I). Although temporal variations in salinity did not show significant correlation with the somatic condition of both sexes, atypical increases in salinity (71.00 in August 2000, 83.6 in April 2001 and 58.67 in March 2002; Figure 1) coincided with minimum predicted M_E values. Moreover, the samplings that showed higher values of submerged vegetation also presented higher values of condition and gonad activity (Table I).

Discussion

In terms of catchability, relative abundance of the population studied showed significant temporal variations characterized by maxima values at the end of summer and early autumn in both years. The decrease of this parameter in spring 2000, a period of recruitment, is rare and probably related to changes in water salinity at deep levels (see below). Pou-Rovira et al. (2004) found similar results in two populations with few other species. Clavero et al. (2006) studying a population of *Aphanius baeticus* where other fish species were abundant found that density peaked in early summer due to the massive emergence of young of the year, and then suffered a sharp decrease suggesting high juvenile mortality rates. Competitive effects upon *Aphanius iberus* juveniles as a result of the presence of other species has been demostrated in microcosm experiments (Rincón et al. 2002; Caiola & De Sostoa, 2005). In the Marchamalo wetland, *Aphanius iberus* only co-exists with a very low-density population of Mugilids (unpublished data), so interspecific competitive effects should be minimal.

Table IV. Final design of ANCOVA analyses of the L_T–M_E and L_T–M_G relationships in *Aphanius iberus* ($P \leqslant 0.05$): F-statistics, degrees of freedom (df) and *P* values. All variables (dependent and covariate) were log-transformed. Total length (L_T) is the covariate.

(a) L_T–M_E relationship

Source of variation	F	df	*P*
Total (test for interaction)			
Length	13,663.44	1, 993	<0.0005
Sex	0.03	1, 993	0.852
Season	8.18	8, 993	<0.0005
Sex × Season	0.54	8, 993	0.823
Length × Sex × Season	4.28	17, 993	<0.0005
Males (test for interaction)			
Length	5,495.14	1, 482	<0.0005
Season	3.50	8, 482	0.001
Length × Season	3.44	8, 482	0.001
Females (test for interaction)			
Length	8,779.23	1, 510	<0.0005
Season	5.57	8, 510	<0.0005
Length × Season	5.67	8, 510	<0.0005

(b) L_T–M_G relationship

Source of variation	F	df	*P*
(i) Total (test for interaction)			
Length	623.58	1, 988	<0.0005
Sex	26.22	1, 988	<0.0005
Season	6.92	8, 988	<0.0005
Sex × Season	0.92	8, 988	0.498
Length × Sex × Season	6.37	17, 988	<0.0005
(ii) Males (test for interaction)			
Length	279.32	1, 482	<0.0005
Season	4.50	8, 482	<0.0005
Length × Season	4.64	8, 482	<0.0005
c) Females (test for interaction)			
Length	383.28	1, 505	<0.0005
Season	3.91	8, 505	<0.0005
Length × Season	4.67	8, 505	<0.0005

The effects of sampling on the studied population (a total of 997 individuals during 2 years) should be considered practically null because its population's estimated size was notably high (summer density in mats=86–131 individuals m^{-2}; unpublished data). Moreover, although CPUEs are often influenced by behavior of fish, without significant migration effects and in absence of better indicators seasonal catchability could be appropriately reflecting the variations of abundance.

Submerged vegetation density strongly governs fish abundance by decreasing predation risk and increasing food supplies (Hindel et al. 2001). In the Marchamalo wetland, submerged vegetation density showed a high temporal correlation with relative abundance in *Aphanius iberus*. This agrees with temporal patterns previously shown for other small fish (Jordan 2002; Lewin et al. 2004). Moreno-Amich et al. (1999) pointed out the importance of submerged vegetation mats, mainly *Ruppia* spp., as habitat for *Aphanius iberus*, and Alcaraz et al. (2008) showed that the density of the species was lower in the open waters than in the glasswort and algal mat habitats. High biomass and density of water column invertebrates ocurred in submerged vegetation mats and the diet shown for the species was based rather on these organisms (Alcaraz & García-Berthou 2006).

Drastic and rapid changes in water salinity coincided with minima values of abundance as detected in other similar habitats (Molony & Parry 2006). High salinity levels may have negative effects on the metabolism of cyprinodontids (Plaut 2000; Oliva-Paterna et al. 2006b), on growth performance and survival (Yildirim & Karacuha 2008), and on other fish species, especially on particular fractions of the populations as larval stages or juveniles

Table V. Comparison of predicted M_E and M_G values of *Aphanius iberus* by ANOVA and Tukey's HSD tests ($P \leqslant 0.05$): F-statistics, degrees of freedom (df) and *P* values. Sp00=predicted M_E mean of Spring 2000, etc.

(a) Predicted M_E values				
Males	ANOVA	F	df	*P*
		30.846	8, 482	<0.001
	Tukey's HSD			
	Sp00=Sm00>Au00=Wi01=Sp01>Sm01=Au01<Wi02<Sp02			
Females	ANOVA	F	df	*P*
		43.670	8, 510	<0.001
	Tukey's HSD			
	Sp00=Sm00>Au00=Wi01=Sp01>Sm01=Au01=Wi02<Sp02			
(b) Predicted M_G values				
Males	ANOVA	F	df	*P*
		46.475	8, 482	<0.001
	Tukey's HSD			
	Sp00=Sm00>Au00=Wi01<Sp01=Sm01>Au01=Wi02<Sp02			
Females	ANOVA	F	df	*P*
		110.319	8, 510	<0.001
	Tukey's HSD			
	Sp00>Sm00>Au00<Wi01<Sp01<Sm01>Au01=Wi02<Sp02			

(Bohlen 1999; Boyce 1999). In general, it is important to detect the optimum salinity level for each fish species thriving in different environments. However, these adverse effects do not contradict the fact that hypersaline systems are important habitats for the conservation of Iberian toothcarps (Gutiérrez-Estrada et al. 1998; Oliva-Paterna 2006).

Coinciding with the first salinity increase (August 2000), massive mortalities were observed in the studied population and also in the low density of Mugilids cohabited population. In confined aquatic systems, collateral effects are known to occur, such as the accumulation of ammonia components, increased vulnerability to disease and pathological changes in gill structure, among others (Wilkie 1997). These effects could be acting on the population studied, aggravated by the decrease approximately 50% in submerged *Ruppia* spp., which was likely due to increased salinity (Sim et al. 2006). These collateral effects could be responsible for the sharp decrease in the relative abundance (CPUEs) and in condition of this population which never recovered the high CPUE values observed during the first spring. In fact, seeing the high correlations found between relative abundance and submerged vegetation, it could not be discarded a covariation of salinity and plant recovery, since high values of salinity may also cause mortality of *Ruppia* spp.

Salt extraction management in Marchamalo presently wetland does not take into consideration the established population of *Aphanius iberus*. For instance, closing the entry channel, produced low water levels which increased salinity considerably. This process coincided with the recruitment period (beginning of summer 2000) and reproduction (spring 2001). It has been observed that extreme salinity reduces survival in *Aphanius iberus* (Sanz 1985) and others Cyprinodontids (Yildirim & Karacuha 2008). Moreover, Oltra and Todolí (2000) speculate that high salinity *per se* does not provide any reproductive advantage.

Maximum total length of females observed in the Alt Empordà wetlands (52.6 mm) and in the Ebro delta (45 mm) were smaller than in the present study (60 mm), partially confirming latitudinal variation of sizes described for fish species (Wootton 1998). The Alt Empordà and Ebro delta are exposed to less predictable environmental conditions and water temperature, turbidity and oxygen can fluctuate rapidly (Vargas & De Sostoa 1997), characteristics which might have determined the smaller fish sizes. In Marchamalo wetland, values of water temperature were always above 15°C except in winter, which is an indicator of the benign environmental conditions where the studied population occurs.

The population structure of *A. iberus* in Marchamalo wetland was formed by three age groups (0+, 1+ and 2+) in both sexes, similar to that observed in the most northerly population located in the Alt Empordà wetlands (García-Berthou & Moreno-Amich 1992). In the Ebro delta, however, the maximum age observed was 1+ (Vargas & De Sostoa 1997).

García-Berthou and Moreno-Amich (1992) showed a high degree of overlap of size groups

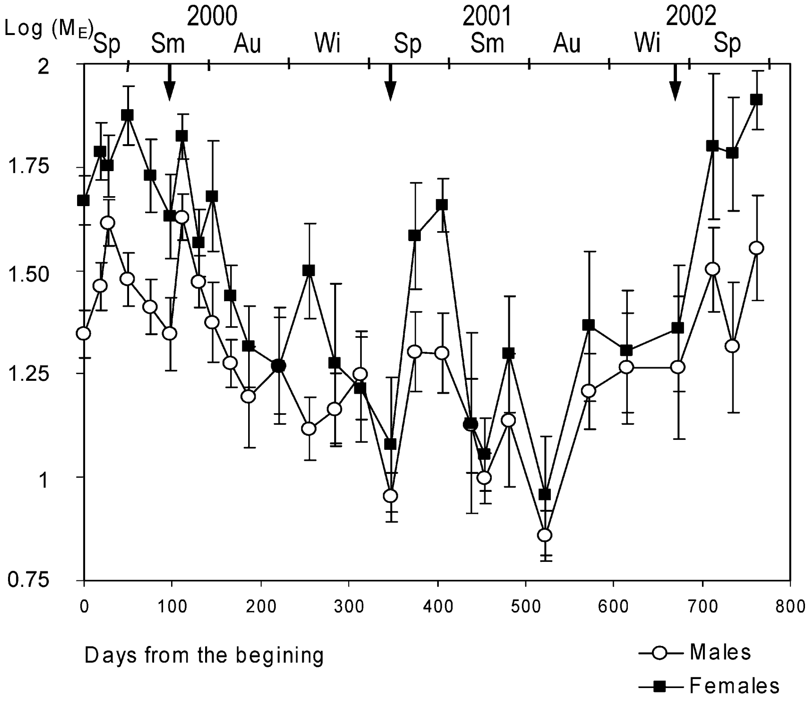

Figure 5. Seasonal changes in somatic condition for mature males and females of *Aphanius iberus*. Mean ± 95% CL of predicted M_E values for samples of 10 or more fish. Marks separate the seasons: (Sp) Spring, (Sm) Summer, (Au) Autumn and (Wi) Winter; black arrows mark maxima in water salinity.

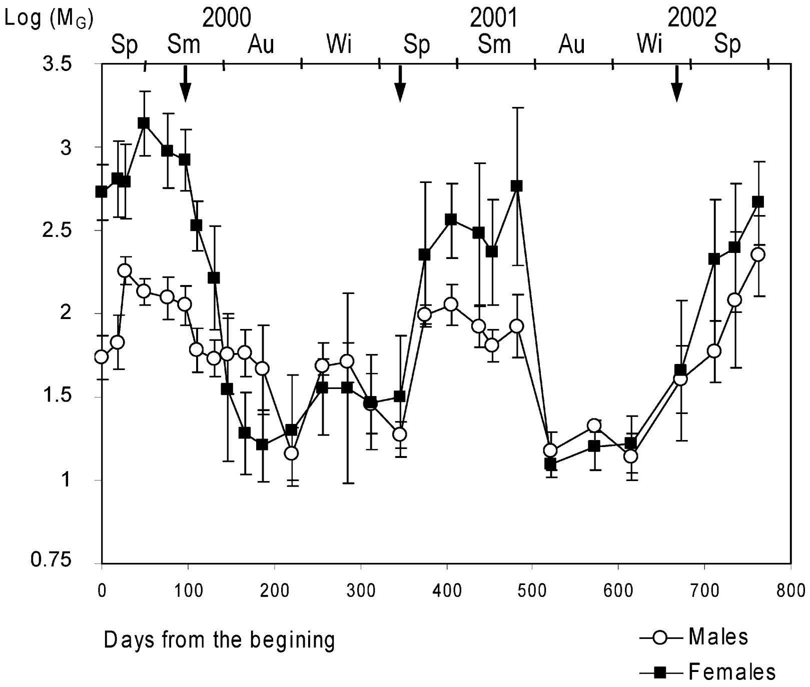

Figure 6. Seasonal changes in gonad activity for mature males and females of *Aphanius iberus* population located in the Marchamalo wetland. Mean ± 95% CL of predicted M_G values for samples of 10 or more fish. Marks separate the seasons: (Sp) Spring, (Sm) Summer, (Au) Autumn and (Wi) Winter; black arrows mark maxima in water salinity.

within the 0+ age class, while in our study the existence of up to three different cohorts (size groups) within the 0+ age class during several seasons was confirmed. This would reflect high growth rates of newborn and younger cohorts.

Sex ratio in Marchamalo wetland depends on size, age and reproductive period as has been observed in other populations (Vargas & De Sostoa 1997). High mortality rates have been detected in the 1+ age class after reproduction (Fernández-Delgado et al. 1988; Vargas & De Sostoa 1997). In the population studied, this phenomenon seems to be more pronounced in females as males were dominant in the 1+ group of seasons after the reproductive periods (winter 2000–01, autumn 2001 and winter 2000–01). In the Marchamalo population there was no massive disappearance of the 1+ group after reproduction as has been detected in other *Aphanius* populations (Fernández-Delgado et al. 1988; Vargas & De Sostoa 1997). Indeed, this age group made up 21.54% of the total of individuals in contrast to the 12% and 13.7% in the Ebro delta and the Alt Empordà wetlands, respectively (García-Berthou & Moreno-Amich 1992; Vargas & De Sostoa 1997). These differences might confirm latitudinal variations due to the more beningn environmental conditions. In turn, the remarkedly higher presence of males in the 1+ age class (66.4% males) is also exclusive to the Marchamalo population in comparison with other populations studied (Vargas & De Sostoa 1997).

In Marchamalo wetland, the recruitment period began in May and lasted until November, constituting the longest period observed for *Aphanius iberus*. This phenomenon along with the presence of small immature fish (<12 mm L_T) in winter, underline the favorable ecological conditions in this wetland because the water temperature is over 11°C all the year. In contrast, the unusual absence of small immature fish in samples within the reproductive period could reflect different survival rates for immature and mature fish, perhaps as a consequence of the drastic increases in salinity. In general, the first stages of life in fish are the most sensitive to environmental stress (Bohlen 1999; Molony & Parry 2006).

Somatic condition was influenced by both environmental factors (water temperature and salinity) and reproductive cycle (Spearman's correlation for predicted M_E and M_G values; Males: $Rs=0.571$, $P=0.002$; Females: $Rs=0.608$, $P=0.001$). The index reached its lowest values at the end of summer and autumn, increasing in winter and reaching maximum values in late spring and early summer, when spawning triggered a decrease in somatic condition. Similar results have been observed in other populations. Maximum summer condition values have been to coincide with higher water temperature and productivity (Vargas & De Sostoa 1997). Although in this study we did not detect this relationship we showed that drastic changes in water salinity caused a decrease in condition of females.

The gonadal development of males and females showed the same pattern with three different phases in gonadal activity (quiescence, gonad growth and reproduction). Gonad growth began in early spring and the reproductive period extended from April/May to August, which is similar to that observed in the Ebro delta (May–August) and Empordà wetland (March–July). During the first year, the quiescence stage of males showed sharp variations of M_G, perhaps as a consequence of drastic changes in environmental conditions, with different effects for males than females.

The presence of multi-modes in length frequency distributions of 0+ and the long reproductive period found in our population agree with the batch spawning strategy characteristic of several *Aphanius* populations (Fernández-Delgado et al. 1988; Vargas & De Sostoa 1997; Leonardos & Sinis 1998). This reproductive period usually coincides with favorable climatic factors, which result in greater food availability and oocyte maturation (De Vlaming et al. 1978). The water temperature in Marchamalo wetland was relatively high during the two years of the study, favoring a long reproductive period. Frenkel and Goren (1997) in *Aphanius dispar*, and Byniak (1979) (in Frenkel & Goren 1997) in *Aphanius mento*, detected positive relationships between temperature and oocyte maturation. In Marchamalo, the gonadal cycle correlated with water temperature, and reproduction coincided with water temperatures >20°C.

In our population, salinity variations seemed to produce occasional upsets in its pattern, especially in males. Although cyprinodontids generally show great adaptability to salinity, this environmental variable has been described as affecting oocyte status in other species (Hoar & Randall 1984), which could explain our results.

In summary, the studied population, located in a hypersaline habitat with a high water temperature all the year, showed the opportunistic strategy mentioned by Vila-Gispert and Moreno-Amich (2002). In the absence of effects derived from migration (in-migration and out-migration) and interspecific interactions, drastic changes in environmental conditions, such as salinity and water level, could be linked to decreases in *Aphanius iberus* abundance. The population structure is characterized by a low number of age groups, a high overlapping of size groups within the 0+ age class and a short life span, but a very long reproductive period and a temporal condition pattern affected by both environmental and reproductive factors.

Oliva-Paterna et al. (2006a) showed how the salt exploitation wetlands are the most important aquatic systems for conservation of *Aphanius iberus* in its southern distribution range. Nevertheless, because the biological criteria are not included in their exploitation, life-history traits of the species are often being affected. In this study we detected changes in abundance, population size structure, somatic condition and gonad activity in relationship with the input–output of water. Changes in water level that increase the salinity should be indirect effects on population dynamic by reducing vegetation mats. Since *Aphanius iberus* is an endangered species (IUCN 2006), these effects should be considered when such populations are exposed to recovery plans and when selecting possible habitats for reintroductions.

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