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Growth and Reproductive Ecology of the Endemic Freshwater Fish *Alburnus vistonicus* Freyhof & Kottelat, 2007 (Actinopterygii: Cyprinidae) in Lake Vistonis System, Northern Greece

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Abstract: We studied the age structure, growth rate and reproductive biology of the local Greek endemic fish *Alburnus vistonicus* Freyhof & Kottelat, 2007, collected in October 2014 - September 2015 from Lake Vistonis, Northern Greece. Sex was identified in 477 (53.4%) females and 416 (46.6%) males. A positive allometry in growth was detected for both sexes: b=3.4827±0.020, t-test P<0.0005 in females and b=3.4944±0.030, t-test P<0.0005 in males. Eight year classes were identified, with 1 and 3 being the dominant (23.9%). The highest value of the asymptotic length L_{∞} (37.56 cm) was estimated for females, while growth parameter K was equal in both sexes (0.15 y^{-1}). Gonadosomatic index (GSI) in females peaked in April and May, while in males the period of testicular growth was restricted between late March and early May. No difference in the size at maturity between males and females was detected, resulting in a length at maturity L50=11.3±0.38 cm for both sexes. The results of the study will provide insight into the population of *Alburnus vistonicus* and could assist the implementation of future management plans in the area for species' protection.

Key words: life history strategies, environmental factors, Vistonis shemaja, salinisation, Greece

Introduction

Vistonis shemaja *Alburnus vistonicus* Freyhof & Kottelat, 2007 is a freshwater fish with a very restricted distribution in the catchments of Lake Vistonis and Lake Ismarida and the Filiouris River in Northern Greece, Southern Balkan Peninsula (Freyhof & Kottelat 2007). The species has been assessed as "critically endangered" (CR) under the IUCN Red List (www.iucnredlist.org). It is protected by the national and international law, and is included in Annex II of the Directive 92/43/EEC and the Red Book of Threatened Species of Greece (Legakis & Maragou 2009). However, despite the strict conservation status, the species is fished and usually captured as bycatch during commercial fishing.

The species biology is poorly known (Kokkinakis 1992). Two populations, originally misidentified as *Chalcalburnus chalcoides macedonicus* in lakes Volvi and Vistonis (Northern Greece) were later confirmed as *Alburnus volviticus* in Lake Volvi and *Alburnus vistonicus* in Lake Vistonis, respectively (Freyhof & Kottelat 2007). Hydromorphological alterations, which have corrupted the continuity between Lake Vistonis and the inflowing rivers used by the species as reproductive areas (Kokkinakis 1992, Bobori et al. 2015), along with salinisation, are threatening the species. As a consequence, the population has declined (Bobori et al. 2015).

So far, several freshwater fish species worldwide have been led to extinction, while others face significant risks (www.iucnredlist.org). Species with restricted distribution and habitat specialisation may be more

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prone to extinction than other common species with a wider range (Sodhi et al. 2009). Thus, understanding species' biology and ecology and particularly their specific life-history attributes is necessary for the development and implementation of effective management plans, enabling ecological monitoring and conservation actions (Mann 1991, Uçkun & Gökçe 2015).

The present study aimed to increase our knowledge on some biology aspects of *Alburnus vistonicus* in Lake Vistonis such as age, growth and reproductive characteristics. The results of the study will provide insight into the population of the species and could assist the implementation of future management plans for the protection of this species.

Materials and Methods

Sampling area

Lake Vistonis is a natural, hypereutrophic, shallow lake (2 m mean depth) with surface area of about 45 km^2. It is located in Northern Greece and its northern part contains low salinity water, due to the freshwater inflows from the Kosynthos, Kompsatos and Travos rivers. The southern part receives the inflows from the Vistonis Gulf via Porto Lagos Lagoon (Koutrakis et al. 2004).

Sampling

Fish sampling was conducted monthly (October 2014 – September 2015), and bi-monthly during May, aiming at a better understanding of the reproductive behaviour of this species. Nordic-type benthic multi-mesh gillnets (Appelberg 2000, CEN 2005) were used.

Fish processing

For each specimen, total length (TL, cm± 0.1), total weight (W, g±0.01) and eviscerated weight (Wev, g±0.01) were measured. Age determination was based upon 10-15 scales removed from the left flank of each fish, above the lateral line and the pectoral fin, and mounted between two slides. The total scale radius (S) and the radii of the different growth rings (S_1, S_2…S_n) were measured along a line in two independent readings. Sex was macroscopically identified. Gonads were weighed (Wg, g) and the gonadosomatic index (GSI=Wg/Wev X 100) was calculated. When sex identification was not straightforward (mainly in fish with very small gonads), sex was identified through observing stereoscopically the gonadal tissue. Males and females were further scored as reproductively active or inactive, based on their size (both sexes) and morphology of the gonads, the latter including colour and homogeneity (only in females). Both males and females with very small, cordlike gonads and females with ovaries containing only primary oocytes (showing to be homogeneous) were scored as inactive, while all other females were scored as active.

Growth

The weight – length relation was calculated using the equation W=aTL^b (LeCren 1951). The resulted *b* values were checked for isometry (*b*=3) by applying one-sample t-test (Zar 1999). Data were then log-transformed for regressions and the null hypothesis of no difference between the slopes was tested (ANCOVA; Zar 1999).

The relative condition factor (RCF) was calculated according to LeCren (1951). The relationship between total scale radius (S, mm) and total length was estimated according to Francis (1990) and, subsequently, the back-calculated lengths at certain age were calculated (Fraser 1916, Lee 1920). Growth parameters were assessed based on both the observed and the back-calculated lengths at age, using the von Bertalanffy growth equation (VBGF, see von Bertalanffy 1938). Using the afore-estimated growth parameters and the mean temperature of the water surface (T, °C), the growth index φ' and the natural mortality (M) of the species were calculated, using the equations: $\varphi'=\log K+2\log L_\infty$ (Munro & Pauly 1983) and $\log M=-0.0066-0.279\log L_\infty+0.6543\log K+0.463\ \log T$ (Pauly 1980), respectively.

Reproduction

The pattern of gonadal allometry for reproductively active and inactive males and females was examined for validating the macroscopic maturity scorings. The log-transformed values of Wg were plotted against log-transformed values of Wev and the plots were visually examined to check whether the scatter points corresponding to values of maturity stage were satisfactorily separated (McPherson et al. 2011). Reproductive activity data were transformed to maturity data by applying a temporal filter to the reproductive peak of the population (Hunter & Macewicz 2003, Lowerre-Barbieri et al. 2011) when the prevalence of mature-inactive individuals was eliminated and the population mostly comprised of immature and mature/spawning-capable fish. Dichotomous maturity data (0: immature; 1: mature) were modelled as a function of TL (continuous variable) and sex (factorial variable) using generalised linear models (GLM), with a binomial error distribution and a logit link. Variable selection was performed through a stepwise backward Akaike's information criterion

(AIC-based process). The analysis led to estimates of length at maturity (L50), i.e. the length at which 50% of the population has become mature. The estimated sex ratio was tested for differences against the 1:1 ratio using chi-square test (χ^2 test, SPSS ver.21).

Results

Age and growth

A total of 893 individuals were caught with total length ranging between 6.3 and 28.8 cm and weights varying from 1.86 to 345.1 g. From those, 477 (53.4%) were females and 416 (46.6%) males, providing a female-male sex ratio equal to 1:0.87 that did not differ statistically from a ratio of 1:1 (χ^2=0.2141, P>0.05). A positive allometry in growth was detected for both sexes (b=3.4827±0.020; t-test, P<0.0005 and b=3.4944±0.030; t-test, P<0.0005, for females and males, respectively), with no statistically significant differences between the slopes of the relationships (ANCOVA F=0.106, P>0.05).

Age and growth analysis was based on 804 out of the 893 specimens caught. Differences between the two scale readings were observed for 12.81% of the scale samples. Eight age classes were identified, with 1 and 3 being the dominant (23.9%; Table 1). The majority of females were 2 years old (21.4%), while males – 4 years old (33.6%). Mean annual growth was higher during the first year (36.6%), followed by a gradual decline, with the exception of year 4 (Table 1).

In general, mean back-calculated lengths at certain age were lower compared to the mean observed when both sexes were considered (Table 1). The estimated von Bertalanffy equations based on the observed TL were: L_t= 37.56(1-$e^{0.15(t+1.15)}$) and L_t= 36.67(1-$e^{0.15(t+1.34)}$) for females and males, respectively, while when the back-calculated lengths at age were used the equations were modelled as L_t=36.61(1-$e^{0.15(t+1.25)}$) for females and L_t=25.36(1-$e^{0.31(t+0.70)}$) for males. Growth index φ′ values for females and males were φ′=2.32 and φ′=2.30, respectively, based on the observed for both sexes when the back-calculated total lengths were considered. Accordingly, mortality was estimated as M=0.40 y^{-1} for both sexes. Finally, the highest mean RCF value was observed in June (0.689), whereas when sexes were considered separately, females were found in a higher condition during November (0.709). However, fish condition did not differ between sexes (P>0.05), while RCF variability was significant when only temporality was considered (ANOVA, F=18.471, P<0.001).

Reproduction

Reproductively active individuals discriminated well from inactive individuals in both male and female specimens (Fig. 1), confirming the accuracy of the macroscopic stage classification. The slopes of the gonad over body weight relationships did not differ significantly between active and inactive fish in both males and females (ANCOVA, P>0.1; Fig. 1), suggesting no shift in the allometric growth pattern of both testes and ovaries during gonadal development.

Individuals above the threshold line (Fig. 2A) were all mature in females and mostly mature in males. Females started allocating energy to ovarian growth in early December, peaking in April and May, while males had a more restricted period of testicular growth between late March and early May (Fig. 2A). The length at maturity was estimated by applying a temporal filter to the seasonal peak of reproduction. Backward stepwise entry in the GLM analysis showed that only length affected maturity, whilst sex was not a significant predictor. Therefore, despite the aforementioned differences in gonadal seasonal-

Table 1. Mean observed and back calculated total lengths (TL, cm) at age (years) for *Alburnus vistonicus* females (♀) and males (♂) in Lake Vistonis. MAI: the Mean Annual Increment (%) in fish size compared to the maximum attainable total length.

Mean observed TL (cm) at age (years)									
	n	1	2	3	4	5	6	7	8
♀	308	10.5±0.18	13.6±0.11	16.6±0.19	20.4±0.20	22.3±0.08	24.8±0.39	26.2±0.20	27.5
		n=53	n=66	n=57	n=56	n=62	n=10	n=3	n=1
%	MAI	10.48	3.07	3.05	3.75	1.96	2.47	1.39	1.33
♂	**295**	11.0±0.22	13.7±0.14	16.6±0.14	20.1±0.13	22.3±0.22	23.2±2.77		28.8
		n=32	n=58	n=87	n=99	n=15	n=3	-	n=1
%	MAI	11.02	2.71	2.87	3.54	2.12	0.91	-	-
Mean back-calculated TL (cm) at age (years)									
	n	1	2	3	4	5	6	7	8
♀	308	10.5±0.05	13.8±0.07	16.8±0.09	19.6±0.10	22.0±0.10	24.1±0.29	25.5±0.27	27.1
		n=308	n=255	n=189	n=132	n=76	n=14	n=4	n=1
%	MAI	10.46	3.38	2.98	2.77	2.42	2.13	1.40	1.56
♂	**295**	10.9±0.04	14.1±0.07	16.9±0.09	19.4±0.12	21.5±0.44	23.2±2.06	26.3	28.2
		n=295	n=263	n=205	n=118	n=19	n=4	n=1	n=1
%	MAI	10.94	3.16	2.78	2.54	2.03	1.77	3.08	1.92

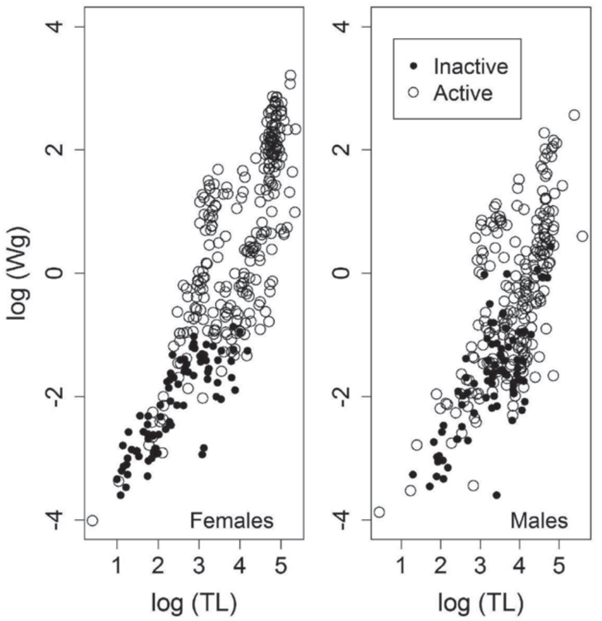
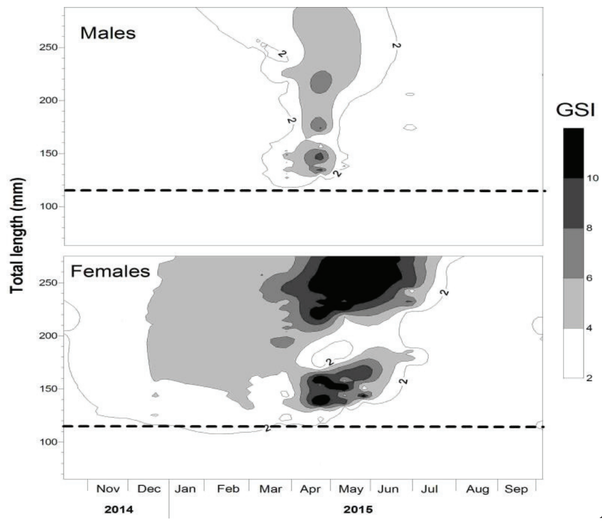


Fig. 1. Relationship between log-transformed gonad weight (Wg) and total length (TL) for the reproductively active and inactive *Alburnus vistonicus* in Lake Vistonis. Dashed horizontal lines correspond to the threshold Wg values between active and inactive females (left panel) and males (right panel).



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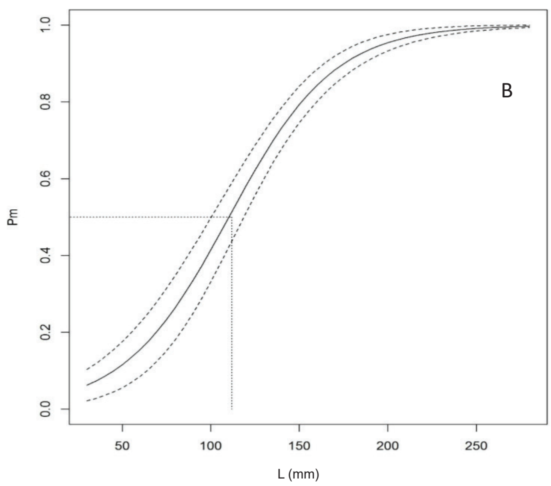

B

Fig. 2. (A) Seasonal evolution of gonadosomatic index (GSI) of male and female *Alburnus vistonicus* in Lake Vistonis per total length. Dashed horizontal lines represent the length at maturity (L50). (B) Maturity ogive, i.e. probability of being mature (Pm) versus body length (L), for males and females of *Alburnus vistonicus* in Lake Vistonis. Dotted lines correspond to 95% confidence intervals. Rectangular segments indicate length at 50% maturity for the two sexes.

ity and growth, logistic GLM analysis showed that there was no difference in the size at maturity between males and females. Overall value of length at maturity for both sexes was 11.3 (±0.38) cm (Fig. 2B). When this value was superimposed to the plot of GSI seasonality with body length (Fig. 2A), it was clear that reproductive activity in both males and females started when GSI switched to values higher than 2%. This simple plot, constructed using simple and routine biometric data such as body length and gonad weight, proved to be very accurate in acquiring some crude measure of length at maturity. As shown in Fig. 2A, at peak spawning months the female GSI can exceed 10%, while there were females with GSI values as high as 23%. On the other hand, male GSI was quite smaller (Fig.2A) and its values during the spawning months ranged between 4-8%.

An abnormal pattern of gonadal growth was observed at peak spawning months in mid-sized females (between 17 and 22 cm). More specifically, while ovarian growth was progressing normally for all size classes during the months that preceded the spawning season (i.e. from January to March), the ovaries of mid-sized females suddenly ceased growing during the spawning season (Fig. 2). This pattern was not observed in males, where gonadal growth was even for all size classes at peak spawning months, apart from a small burst of GSI between 15 and 17 cm TL. These abnormal individuals, i.e. females and males above the length at maturity with GSI values lower than 2% at peak reproduction, showed lower relative condition factor compared to reproductively active individuals during all peak spawning months (Fig. 3).

Discussion

In the present study, a positive allometric growth was evident for *Alburnus vistonicus*; however, *b* values calculated by us were lower than those reported by Kokkinakis (1992). It is known that several factors, including biological and environmental pa-

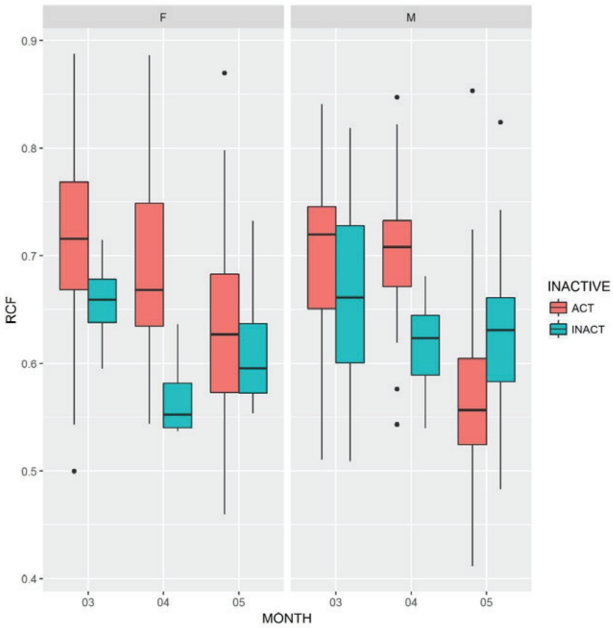

Fig. 3. Monthly variation of relative condition factor (RCF) of reproductive active (ACT) and inactive (INACT) females (F) and males (M) of *Alburnus vistonicus* in Lake Vistonis during the reproductive peak.

rameters, could affect WLRs (LeCren 1951, Pauly 1980, Froese 2006), while a bias in the results could be introduced by the used sampling gear (Froese 2006). Indeed, the size of gill nets used in the present study (5-55 mm) differed from the net sizes used by Kokkinakis (1992) (14-50 mm), resulting in differences in the size range of the caught individuals (present study – 6.3-28.8 cm; Kokkinakis (1992) – 9.8-24.41 cm). Nevertheless, parameter *b* provides useful information on fish growth and usually varies between 2.6 and 3.4 (Froese et al. 2011). Our *b* estimations were a little higher than the upper limits of this range but within the range estimated for most freshwater fish species in Greece (2.14-3.70; Kleanthidis et al. 1999). Generally, the population of *A. vistonicus* was dominated by the younger age classes (I-IV; 87.5%), indicating a healthy population (Brunel & Piet 2013), which is harvested (Hunter et al. 2015) at ages older than IV years, at sizes greater than 20 cm. However, several other factors, such as natural mortality, competition, predation, food availability, abiotic environment, fishery, could cause the observed decrease in the number of fish in older ages.

Alburnus vistonicus seemed to follow the general growth pattern (Wootton 1992). However, between age classes 2 and 4, an increment in the mean annual growth was observed, followed by a decrease in the subsequent years. Changes in the dietary habits can affect, among others, fish growth (Wootton 1992). Indeed, in the case of *A. vistonicus*, an alteration in species trophic preferences from planktivory to invertivory was evident under the 2 to 4 age classes (Bobori et al. 2015), resulting in this differentiation in length growth pattern.

The length back-calculation method is widely used in fisheries biology and may provide useful data for a data-poor fish stock or even re-confirm the results from the existing ones (Francis 1990). In the present study, this method was used in order to verify the values of the growth parameters estimated using the observed total lengths (Graynoth 1987). Growth parameters, based on both observed and back-calculated lengths were quite similar but lower than the previously reported (Kokkinakis 1992), possibly due to differences in the methodologies used, since Kokkinakis (1992) applied the Ford–Walford equation. Generally, the asymptotic length estimated for females was higher than in males, reflecting growth differences between the two sexes (Froese & Binohlan 2000). Moreover, the higher growth index φ' for females is indicative of their slightly better ability for growth (Munro & Pauly 1983).

Estimating natural mortality (M) is of particular interest for the management of fish populations (Blanck & Lamouroux 2007). In the present study M did not differ between sexes. However, in a fish population, M may show great variability (Lorenzoni et al. 2002) and may be affected, among others, by species growth (Pauly 1980, Wootton 1992).

The results of the reproductive analysis suggest that the main reproductive season for the population of Lake Vistonis is between April and May. In that respect, males seem to allocate energy to gonadal growth only during the reproductive season, while females begin to allocate energy on gonadal growth quite earlier than the onset of the reproductive season. The most striking difference between the two sexes was the abnormal pattern of gonadal growth, which was observed at peak spawning months in mid-sized females, which were shown to cease ovarian growth. These abnormal individuals showed lower relative condition factor compared to reproductively active individuals during all peak spawning months. It is thus assumed that these individuals failed to go on with allocating energy to reproduction due to restricted or even depleted energy reserves.

In conclusion, even if *A. vistonicus* is not an economically important fish species, it constitutes an important link in the food web of Lake Vistonis, serving especially as food for bird populations (Daoutopoulos 1990). The outcomes of the present study provide insight into the population of the species for implementation in future management plans in the area, focusing on its protection. Therefore, priority should be given in ensuring successful reproduction of this species by maintaining the continuity of river habitats with the lake (e.g. removing barriers), thus facilitating the free movement of the species. The implementation of such plans will contribute to the long-term existence of the species in its natural range and will enable the empowerment of its populations.

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