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Age and growth of Arno goby, *Padogobius nigricans* (Canestrini, 1867), in the Aggia River (Umbria, Central Italy)

By L. Pompei¹, D. Giannetto² and M. Lorenzoni¹

¹Department of Chemistry, Biology and Biotechnology, University of Perugia, Perugia, Italy; ²Department of Biology, Faculty of Sciences, Mugla Sitki Kocman University, Kotekli/Mugla, Turkey

Summary

In this study, data of Padogobius nigricans, a species endemic to Central Italy, were used to assess the age, growth parameters and condition of this species. Length-weight relationships are also provided and represent the first available reference in the literature for the Arno goby. Data were collected in the Aggia River, a tributary of the Tiber River, by monthly samplings. A total of 780 specimens ranging in size from 1.5 to 9.2 cm were analyzed. Age was assessed by examination of scales, with the maximum age of 4+ years observed in males and 3+ in females. Previous growth was determined by back-calculation of scale measurements. The von Bertalanffy growth parameters were estimated separately for sexes (males: $L_{\infty} = 9.759$ cm, k = 0.505, $t_0 = 0.025$, $\Phi' = 1.682$; females: $L_{\infty} = 8.989$, k = 0.458, $t_0 = 0.028$; $\Phi' = 1.599$). Evidence of growth differences between sexes was found, with males living longer and reaching larger sizes. Using the relative condition factor, condition was lower in females than in males, especially during the breeding period due to the strain of high reproduction. Competition with the congeneric P. bonelli, a species endemic to Northern Italy that was introduced in Central Italy, seems not to have been disadvantaged directly by the growth of P. nigricans in the Aggia River, since no selective size-mortality occurred in the population.

Introduction

The Arno goby, Padogobius nigricans (Canestrini, 1867), is endemic in the Tyrrhenian catchment basins of Tuscany, Umbria and Latium (Bianco, 1995). In the Umbria region the species is widely distributed in the northern part of the Tiber River basin and in its major tributaries, with the exception of the Nera River (Lorenzoni et al., 2010). Due to several anthropogenic impacts such as river habitat modifications, water extraction, and pollution, the populations are in sharp decline throughout their range of distribution (Crivelli, 2006). Another threat to their survival is by competition with the Padanian goby, Padogobius bonelli (Bonaparte, 1846), a species endemic to northern Italy and introduced in the early 80s to some watercourses in central Italy as a result of restocking activities (Bianco and Ketmaier, 2001). Regarding the Tiber River basin, P. bonelli was recorded for the first time in 1996 in the Aggia and Cerfone rivers (Mearelli et al.,

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1996), which are small right-tributaries to the Tiber River. From there, in recent years it colonized the upper and middle course of the Tiber River, and is now spreading further into several tributaries (Lorenzoni et al., 2007). Males of the two species fight for occupation of the best breeding sites: P. bonelli shows a more territorial and aggressive behaviour and tends to drive P. nigricans away, preventing its reproduction (Zerunian, 2004). In some river basins (Amaseno and Mignone) of central Italy, P. bonelli have already caused local extinctions of P. nigricans (Nocita and Zerunian, 2007). For these reasons, P. nigricans was assessed as 'vulnerable' according to the IUCN Red List of Threatened Species (Crivelli, 2006) and the Red List of Italian Vertebrates (Rondinini et al., 2013). It is also listed in Annex II of the European Union Habitat Directive 92/43/EEC and in Appendix III of the Bern Convention Council of Europe (1979).

Despite its conservation value, information on the biology and ecology of P. nigricans is still lacking; the only quantitative data available on life history traits of this species regard a population in the Farfa River (Latium) (Scalici and Gibertini, 2009). Gathering valuable information on the biology of the species is crucial for formulation of the management strategy and conservation of P. nigricans populations. Thus, the main intention of this study was to deepen some aspects of the biology of this species and increase the knowledge of its population status and dynamic properties by investigating the Aggia River population. Specifically, the study aim was to: (i) analyze age and growth of the population; (ii) evaluate the physiological condition of the specimens; (iii) assess the presence of any selective size-mortality that occurred in the population; and (iv) provide the first references in the literature for the length-weight and length-length relationships of the species.

Materials and methods

Study area

The Aggia River ($43^{\circ}24'8.57''N$; $12^{\circ}12'40.34''E$) is a small right tributary of the Tiber River, which is the third-longest (405 km) river in Italy and second in watershed area ($17 375 \text{ km}^2$) (Lorenzoni et al., 2006). The Aggia River is 15.5 km long and has poor flow rate (just over 10 L s^{-1}) (Lorenzoni et al., 2010); a Mediterranean climate with maximum rainfall in autumn and minimum in summer characterizes this

biotope, thus the creek has a typical torrential regime and tends to dry up in the summer (Pompei et al., 2014). The riverbed consists essentially of blocks and cobbles, alternating with areas of mud and fine sand in the downstream section. According to the Extended Biotic Index (Ghetti, 1986), which is one of the most widely applied methods of evaluating water quality in Italy using macro-invertebrates as environmental indicators, the river is characterized by high/good quality waters. In addition to P. nigricans, the other main species in the stream include: Tiber barbel Barbus tyberinus Bonaparte 1839, cavedano chub Squalius squalus (Bonaparte 1837), brook chub Squalius lucumonis (Bianco 1983), South European roach Rutilus rubilio (Bonaparte 1837), and brown trout Salmo trutta (Linnaeus 1758) among the native species; and Padogobius bonelli, South European nase Protochondrostoma genei (Bonaparte 1839), and gudgeon Gobio gobio (Linnaeus 1758) among the exotic species (Lorenzoni et al., 2010).

Collection, processing of samples and statistical analysis

Fish were collected monthly from January to December 2012 by electrofishing. The samples were immediately anesthetized with 2-phenoxyethanol and preserved in 4% formaldehyde for laboratory analysis.

Total body length (TL) and standard length (SL) of specimens were measured to the nearest 0.1 cm; individual total weight (W) was recorded to the nearest 0.1 g before dissection. For age determination, scale samples were removed from dorsolateral or ventrolateral rows of the caudal peduncle of each individual (Miller, 1975) and stored in 33% ethanol. Fish scales were observed under a stereomicroscope and the number of *annuli* was counted; two independent age determinations were made by two different operators. When the results differed, an additional determination was made. The microscopic scalimetric method was validated by means of length-frequency distribution (Bagenal, 1978).

The sex was determined through macroscopic observation of the gonads; the gonads were then removed and weighed to the nearest of 1×10^{-3} g (*W*g).

Sex ratio was investigated testing deviation from the 1 : 1 null hypothesis through chi-square (χ^2) analysis for the total sample and separately for each age class.

The relationship between SL and TL (SL-TL) was established for the total sample and separated by sexes using linear regression analysis. The 95% confidence limits (CL) of aand b were estimated. Analysis of covariance (ANCOVA) was used to test differences between sexes.

The relationship between total length and weight was calculated for the total sample and separately for males and females using the expression:

$$W = a T L^b$$

where a is the intercept of the regression and b the slope or regression coefficient. Parameters a and b were estimated by logarithmic linear regression analysis (Froese, 2006): Presence of outliers was verified graphically using $\log_{10}L - \log_{10}W$ plots and eliminated prior to calculations (Froese, 2006). Additionally, 95% confidence limits (CL) of *a* and *b* were estimated. Standard error was calculated for the slope *b*: the hypothesis of isometric growth was tested using Student's *t*-test.

Growth of previous specimens was determined by backcalculation from scale measurements. Back-calculated lengths (BCLs) were estimated for 270 specimens, 124 females and 146 males, by reading four scales from each individual. The scale radius (Sr) from the centre of ossification to the anterior edge of the scale along the oblique direction as well as the radius of the age rings (St) in the same direction were measured for each scale (± 0.01 mm) (Bagenal, 1978) using an image-analysis system (IAS 2000, 1994). Measurement means of Sr and St were considered in the analysis. The relationship between total body length and scale radius was examined for males and females separately through linear regression modelling (Devries and Frie, 1996):

TL = a + bSr

Length at age was back-calculated following the Fraser-Lee model (Bagenal and Tesch, 1985) as:

$$Lt = a + StSr^{-1}(TL - a)$$

BCLs at age were determined for males and females separately; differences between sexes were analyzed by Multiple Analysis of Variance (MANOVA).

To determine whether Lee's Phenomenon was occurring, the Mann–Whitney U-test was used to compare BCLs reached at various *annuli* for each age-group. Lee's phenomenon might be occurring when BCLs at age are smaller for older fish than for younger fish in the sample. For example, this could imply that slower-growing members of the yearclass escaped fishing-related or natural mortality better than did faster-growing members (Devries and Frie, 1996); by contrast, a reverse Lee's phenomenon occurs when BCLs at the various ages are greater for the older fish than for the younger ones in the sample.

Growth was assumed to be described with the von Bertalanffy growth model (VBGM) (von Bertalanffy, 1938):

$$TL_t = L_{\infty} \{1 - \exp[-k(t - t_0)]\}$$

where TL_t is total length (cm) at age t, L_{∞} is theoretical maximum length (cm), k is a constant expressing the rate of approach to L_{∞} and t_0 is the theoretical age at which $TL_t = 0$. The analysis was conducted using values of BCLs from the last *annulus*. BCLs for all *annuli* are commonly used in growth studies; however, in cases where a Lee's Phenomenon occurs, equation parameters values may be over- or underestimated (Vaughan and Burton, 1994). The index of growth performance (Φ') was calculated with the equation of Pauly and Munro (1984):

$$\Phi' = \log_{10} k + 2\log_{10} L_{\infty}$$

where k and L_{∞} are the VBGM parameters; this index enables comparison of growth from different populations within the same species.

The body condition of the specimens was evaluated using the relative condition factor (Le Cren, 1951) expressed by the formula:

 $Kn = W(aTL^b)^{-1}$

where a and b are, respectively, the intercept on the y axis and the coefficient of the LWR calculated on the entire sample. Relative condition factor was also calculated using the somatic weight:

$$\mathbf{K}\mathbf{s} = (W - W_{\mathbf{g}})(a\mathbf{T}\mathbf{L}^b)^{-1}$$

where Wg is the gonad weight.

The trend of both parameters throughout the year was analyzed for males and females separately.

Results

A total of 780 *P. nigricans* specimens from the Aggia River were collected, which ranged in size from a minimum of 1.50 cm to a maximum of 9.20 cm, with the most dominant classes from 4.00 to 5.50 cm (Fig. 1), and weights from 0.038 to 11.00 g. Five age-classes were found (0+ to 4+), with most specimens belonging to the 1+ (n = 457) and to 2+ (n = 209) age-classes. Juveniles were also quite numerous (0+: n = 67), along with a few older specimens (3+: n = 37; 4+: n = 10).

The total sample composed 389 males, 326 females and 65 juveniles. Males were longer and heavier than females. The differences between sexes proved to be highly significant in Student's *t*-test both for length and weight (TL: t = 5.254, P < 0.01; W: t = 4.321, P < 0.01).

The sex ratio observed in the total sample (Table 1) was 1.16 : 1 (M : F), with a significant difference in chi-square analysis ($\chi^2 = 5.551$, P < 0.05). However, this ratio changed significantly with age. In the younger age-classes (0+ to 2+) this was well balanced and the deviation from the expected 1 : 1 ratio was not significant in the chi-square test (Table 1), whereas among older specimens, males predominated and all specimens that had lived through more than four winters were males.



Fig. 1. Total length (TL) frequency distribution, *P. nigricans* (N = 780), collected in Aggia River, 2012, using 1-cm TL interval. White = juveniles; black = males; grey = females

Table 1

P. nigricans age composition captured in Aggia River, 2012, expressed as the number (N) and percentage (%) of male and female specimens in each age-class. Sex ratio (Males : Females) for each age class is indicated. Deviations from unity in sex ratios tested by chi-square test (χ^2), with values of P < 0.05 considered significant

Age class	Males N (%)	Females N (%)	M : F	χ^2	Р
0+	16 (50.00)	16 (50.00)	1:1	0.000	1.000
1+	218 (51.05)	209 (49.05)	1.04 : 1	0.190	0.663
2+	113 (54.07)	96 (45.93)	1.18 : 1	1.383	0.240
3+	32 (86.49)	5 (13.51)	6.40:1	19.703	0.001
4+	10 (100.00)	0 (0.00)	1:0	10.000	0.002
Total	389 (53.86)	326 (46.14)	1.16 : 1	5.551	0.018

Details of minimum and maximum TL, estimated parameters *a* and *b* of the LWR and their 95% confidence limits and coefficients of determination (r^2) for the total sample, males and females are summarized in Table 2. For each of the LWRs, the regression coefficient *b* proved to be statistically >3 at the *t*-test (for females, males and total sample: P < 0.05), indicating a positive allometric growth (Ricker, 1975). All LWRs were highly significant (P < 0.01), with r^2 values >0.960. Moreover high differences in significance emerged between the two sexes at ANCOVA (F = 5.634; P < 0.05), with males significantly heavier than females of the same TL.

In addition, the SL-TL relationships of the total sample, males and females, along with the estimated parameters of the regressions and the coefficient of determination (r^2) are presented in Table 2. All SL-TL relationships were highly significant (P < 0.01) and the coefficients of determination values were 0.995. Comparison between the regressions did not reveal any statistically significant differences between sexes (ANCOVA: F = 1.528, P > 0.05).

The relationship between scale radius and total length of specimens at the time of capture was calculated separately for sexes as TLc = 0.413 + 50.119 Rs ($r^2 = 0.893$, r = 0.945, P < 0.01) for males and TLc = 0.647 + 46.812 Rs ($r^2 = 0.842$, r = 0.918, P < 0.01) for females. Thus, the mean BCLs at age were determined separately for males (Table 3) and females (Table 4). Males were larger than females among year-classes (Fig. 2), and comparison of BCLs between sexes revealed highly significant differences at MANO-VA (F = 10.141; P < 0.01).

Comparisons using the Mann–Whitney *U*-test between mean BCLs of specimens of age t and those of t + 1 did not reveal any significant differences between males or females (Table 5), proving the nonoccurrence of Lee's phenomenon in the *P. nigricans* population of the Aggia River.

BCLs at various ages were used to evaluate the growth specimens. Estimated parameters of VBGM were $L_{\infty} = 9.759$ cm, k = 0.505, $t_0 = 0.025$ for males and $L_{\infty} = 8.989$, k = 0.458, $t_0 = 0.028$. The index of growth performance (Φ') was greater in males (1.682) than in females (1.559).

The mean relative condition factor value calculated for the overall sample was 1.001 ± 0.004 , while the somatic condition factor (Ks) was 0.985 ± 0.004 . The mean value

Table 2

Descriptive statistics of total length (TL in cm) and estimated parameters of standard length-total length (SL-TL) and total length-weight (LWR) relationships for all *P. nigricans* males (M) and females (F) collected in Aggia River, 2012. Sample size (n), minimum (Min) and maximum (Max) total length, SL-TL and LWR parameters (*a*, *b*), their 95% confidence limits (95% CL) and coefficient of determination (r^2) provided

		TL (cm)		Regression parameters				
	n	Min	Max	a	b	95% CL of a	95% CL of b	r^2
LWR								
Total	773	2.50	9.20	0.0114	3.0799	0.0109-0.0120	3.0492-3.1107	0.980
Μ	389	3.00	9.20	0.0111	3.0891	0.0103-0.0191	3.0461-3.1321	0.981
F	326	3.00	7.70	0.0116	3.0863	0.0105-0.0127	3.0192-3.1420	0.968
SL-TL								
Total	780	1.50	9.20	0.2506	1.1565	0.2255-0.2756	1.1505-1.1624	0.995
Μ	389	3.00	9.20	0.2917	1.1484	0.2542-0.3292	1.1400-1.1568	0.995
F	326	3.00	7.70	0.2636	1.1533	0.2176-0.3096	1.1421–1.1645	0.995

Table 3

Mean back-calculated lengths (BCLs) in cm \pm standard error (SE) at successive annuli in male *P. nigricans* captured in Aggia River, 2012. Mean BCLs derived from last annulus shown in bold

Males: Mean BCLs Age class	\pm SE at successive N	annuli 1	2	3	4
1+	81	3 195 + 0 042			
2+	44	3.156 ± 0.060	5.658 ± 0.083		
3+	20	3.313 ± 0.091	5.818 ± 0.119	$\textbf{7.392} \pm \textbf{0.139}$	
4+	1	$2.941 \pm$	$4.750 \pm$	$6.937 \pm$	8.254
Mean \pm SE	146	3.191 ± 0.032	5.693 ± 0.069	7.370 ± 0.134	8.254

Table 4

Mean back-calculated lengths (BCLs) in cm \pm standard error (SE) at successive annuli in female *P. nigricans* captured in Aggia River, 2012. Mean BCLs derived from the last annulus are shown in bold

Females: Mean BCLs \pm SE at successive annuli							
Age class	Ν	1	2	3			
1+	88	3.194 ± 0.044					
2+	33	3.123 ± 0.057	5.289 ± 0.093				
3+	3	2.875 ± 0.159	5.120 ± 0.166	6.614 ± 0.152			
Mean \pm SE	124	3.167 ± 0.035	5.275 ± 0.086	6.614 ± 0.152			

of Kn was higher in the female sample (Kn = 1.024 ± 0.007) than in males (Kn = 0.994 ± 0.005), with highly significant differences in Student's statistical *t*-test (*t* = 3.557, P > 0.001. Condition appears to be affected by the ovary mass, since when comparing the Ks, the mean values for females (Ks = 0.982 ± 0.006) were slightly lower than for males (Ks = 0.988 ± 0.005); however, the differences were not significant statistically (*t* = 0.536, P = 0.594).

Analysis of the trend of Kn and Ks throughout the year (Fig. 3) showed the best condition attained in autumn in both sexes, with the two values gradually decreasing, much more strongly in females, until the end of winter. During spring and summer the values of Kn and Ks in males remained relatively high, then decreased in September, reaching the lowest average values (Kn = 0.947 ± 0.028 ;



Fig. 2. Mean back-calculated length-at-age (BCLs), *P. nigricans* males (solid line) and females (dotted line) collected in Aggia River, 2012. Sample size (N) used for each age class analysis is indicated. Vertical bars = 95% confidence intervals

 $Ks = 0.944 \pm 0.028$). In females, particularly low Ks values were observed from March (Ks = 0.888 \pm 0.018) to June (Ks = 0.890 \pm 0.011); the differences between Kn and Ks were greater from April to July, testifying to the sharp increase in weights during the reproductive period due to the development of the ovaries.

		Age t	Age t		Age $(t + 1)$		Mann–Whitney U-test	
	Age class	n	TL Mean ± SE	n	TL Mean ± SE	Z	Р	
Males	1+	81	3.195 ± 0.042	65	3.127 ± 0.050	0.557	0.577	
	2+	44	5.658 ± 0.083	21	5.767 ± 0.124	0.575	0.565	
	3+	20	7.392 ± 0.139	1	$6.937 \pm$			
Females	1+	88	3.194 ± 0.044	36	3.102 ± 0.055	0.886	0.375	
	2+	33	5.289 ± 0.093	3	5.120 ± 0.166	0.429	0.668	

Table 5 Lee's phenomenon in male and female *P. nigricans* captured in Aggia River, 2012: comparison of back-calculated total lengths between specimens at age *t* and (t + 1) for each age class. Results of Mann–Whitney *U*-test are given; values of P < 0.05 considered significant



Fig. 3. Monthly variation of relative condition factor, male (a) and female (b) *P. nigricans* collected in Aggia River, 2012. Black circle and solid line = condition calculated using total body weight (Kn); white square and dotted line = condition calculated using somatic weight (Ks). Sample size (N) for each sampling month indicated. Vertical bars = 95% confidence intervals

Conclusions

The lifespan of the Aggia River *P. nigricans* proved to be over 4 years, slightly higher than reported by Scalici and Gibertini (2009), who observed a total of four age-classes (0+ to 3+) for both sexes; however, few 4+ age-class individuals were found.

The sex ratio of the entire sample is heavily biased in favour of males, in contrast to the finding of Scalici and Gibertini (2009), who reported a disproportion in sexes, with a population dominated by females. Research on the biology of gobies showed that females outnumbered males (Marconato et al., 1989; Pampoulie et al., 1999; Azevedo and Simas, 2000; Malavasi et al., 2005; Filiz and Toğulga, 2009; Gutowsky and Fox, 2011; Gkenas and Leonardos, 2012; Grul'a et al., 2012): according to Miller (1984), the preponderance of females is very common among gobioid fishes due to a higher nestattaching behavior of males, and a consequently lower susceptibility to capture. Moreover, since males are territorial in their refuge defense, other males are forced to migrate in search of alternative shelter (Scalici and Gibertini, 2009) and may undergo a sharp decrease in numbers. However, in the population under consideration, the sex ratio was well balanced up to 2 years of age; older specimens were almost all males, which proved them to be longer-lived than females.

Estimated b values of the LWRs calculated for the total sample and for males and females separately, was significantly higher than 3 (indicating an isometric growth) in a *t*-test. According to Froese (2006), only when length-weight estimates reasonably cover geographic and inter-annual variation is it possible to discuss isometric versus allometric growth of a species as a whole by using the value of *b*. Nevertheless, given the lack of literature references dealing with LWRs for this species (Froese and Pauly, 2014), the information is highly important for fisheries research and will be useful in increasing basic knowledge of the population ecology in this species (Froese, 2006).

The Aggia River P. nigricans population displayed an evident between-sexes difference in growth. Highly significant differences emerged between the two sexes when comparing the TLW regressions, as males were heavier at a given TL than females. As for the growth pattern, the value of k of the VBGM, which describes the growth rate, testified to a quicker growth in males, which can reach a higher asymptotic length (L_{∞}) . The growth performance index (Φ') result was higher in males than in females. Moreover, by comparing the BCLs at various ages, males were longer than female at all ages; these differences in lengths increased with age. The characteristics are typical of the species (Gandolfi et al., 1991), since acquisition of a larger size might favour males in the intraspecific competition of mate choice by females (Zerunian et al., 1988; Bisazza et al., 1989; Scalici and Gibertini, 2009). The results of the present study are in line with those of Scalici and Gibertini (2009), who found a similar growth pattern in a P. nigricans population in the Farfa River.

As a whole, females presented a better physiological condition, but the highest weight of the ovaries when compared to testes had an effect on the measurement of Kn. Indeed, considering the somatic condition (Ks), no differences emerged between sexes in the actual state of wellness. More information about reproductive investment derived from the monthly trend analyses of Kn and Ks: in females, months in which the difference between the two parameters was more evident testifies to the increase in ovary mass. From this, we can gather that the reproductive period of *P. nigricans* from Aggia River occurred in May and July. In males, the average values of the two indices (Kn and Ks) are quite similar throughout the year, showing a poor reproductive investment.

Best condition was observed for both sexes in autumn in conjunction with resumption of the rains after a period of strong summer drought, with a significant reduction of the flow (Pompei et al., 2014). The rivers of the Mediterranean are subject to large seasonal variations with periods of very low water in summer, which represent a particularly critical phase for survival (Pompei et al., 2011). The improvement in the condition of specimens in autumn compared to September is probably due to the re-establishment of environmental conditions suitable for *P. nigricans*, which require well-oxygenated waters and moderate water flow (Zerunian, 2002). P. nigricans are penalized by low temperatures and inadequate food from January to March (Pompei et al., 2014). As of March, reproduction also entails a high expenditure of energy that results in a sharp decline of somatic conditions, with the Ks values remaining low until June. The mortality of older females shown by the unbalanced sex ratio characterizing the Aggia River population could be caused by a particularly high reproductive strain. The ensuing post-spawning debility might render a fish more likely to die from outside forces (Miller, 1975), with females unable to accumulate the energy reserves that were required when addressing the preceding period of reproduction.

Comparison among the BCLs excluded a selective sizerelated mortality phenomena in the Aggia River population; in particular, the absence of a reverse Lee's phenomenon in both sexes, which occurs when larger specimens for a given age class survive, highlights the lack of a selective mortality of smaller individuals that would be expected with competition. One of the primary sources of competition for P. nigricans is certainly represented by the cogeneric P. bonelli, which could prevent the reproduction of P. nigricans by taking over all available shelters. Moreover, both are benthic and small-size species that share a very similar trophic niche (Pompei et al., 2014). However, the presence of P. bonelli does not seem to have a direct disadvantage on P. nigricans growth in the Aggia River. Further investigation on the biology and ecology of both P. nigricans and P. bonelli will certainly help to deepen some aspects of the interaction between the two species.

The results increase the knowledge of growth rates and population dynamics of this endemic Italian species. The study provides a framework for further and deeper investigations on the ecology of *P. nigricans* and will be useful to improve conservation actions and monitoring activities for preservation of the populations.

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- Author's address: Laura Pompei, Department of Chemistry, Biology and Biotechnology, University of Perugia, Via Elce di Sotto, I-06123 Perugia, Italy. E-mail: laura.pompei@studenti.unipg.it