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# Analysis of the biological features of the goldfish *Carassius auratus auratus* in Lake Trasimeno (Umbria, Italy) with a view to drawing up plans for population control

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**Abstract.** The growth and reproductive biology of the invasive goldfish *Carassius auratus auratus* were studied in Lake Trasimeno, central Italy. The results of the research revealed that the population is made up of eight age-classes. The *sex ratio* proved to be extremely unbalanced (1 : 19 in favour of females). Growth can be deemed rapid, the von Bertalanffy growth in length function being  $TL = 43.019 \{1 - e^{(-0.272(t+0.162)}\}$  for the total sample and  $\phi' = 2.702$ . No sexual dimorphism in growth was observed. Back-calculation analysis suggested the existence of an inverse Lee phenomenon among 1 year-old specimens. The reproductive period covers a broad time-span, from March to June. In females, sexual maturity is reached after the second winter of life (2+ age-class); however, a small percentage (7.55%) of females is able to reproduce at the age of 1 year. Most of the males attained sexual maturity in the first year (60.61%). The reproductive investment of the females is high; the relationship between SL and the number of eggs was  $N_e = 0.0041 SL^{4.368}$ . Fecundity varied from 286 to 219104 eggs, with an average relative fecundity of  $103 \pm 5$  eggs  $g^{-1}$ ; the mean diameter of the eggs was  $1.27 \pm 0.01$  mm. The reproductive investment of the females was not homogeneous across the age-classes; in addition to absolute fecundity, relative fecundity and egg diameter were seen to increase with the size of the specimens.

**Key words:** growth, *sex ratio*, age structure, fecundity, condition factor

## Introduction

The goldfish *Carassius auratus auratus* (Linnaeus, 1758), a cyprinid native to Eastern Asia (Lelek 1987), is an example of a non-indigenous fish species that has successfully established populations throughout Europe (Kottelat & Freyhof 2007), North (Jenkins & Burkhead 1993) and South America (Gomez et al. 1997), New Zealand and Australia (Department of Fisheries of Western Australia 2005). There has been considerable confusion concerning the taxonomic status of the *Carassius auratus* complex, which comprises a set of different taxa (Vetešník et al. 2007, Papoušek et al. 2008), the position of which is somewhat uncertain (Hanfling et al. 2005). The

distribution of *C. a. auratus* in Europe is not completely clear, since data probably include many populations of the Prussian carp *Carassius gibelio* (Bloch, 1792), which was previously regarded as a feral form of *Carassius auratus* (Kottelat & Freyhof 2007). The two taxa are usually confused; this is also the case in Italy, where the checklist of fish species does not include the Prussian carp (Gandolfi et al. 1991).

The goldfish can spread spontaneously through the connections of hydrological networks, and its range of distribution in Europe is currently expanding; it easily becomes one of the dominant species in stagnant and slow-running waters. Moreover, it is known to be one of the most hazardous species

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for native fish communities (Crivelli 1995). The principal threat to indigenous fish species is probably competition for food and other resources. It has been reported that the goldfish introduced into Europe affect resident fish, such as the carp (*Cyprinus carpio* L.), crucian carp (*Carassius carassius* L.) and tench (*Tinca tinca* L.) (Halačka et al. 2003); in addition, pike (*Esox lucius* L.) may decline in abundance as a result of increased water turbidity (Cowx 1997).

The fish population in Lake Trasimeno, as in the rest of the Tiber River basin, is compromised by the presence of exotic species (Lorenzoni et al. 2006). Management of the introduced species is often crucial to maintaining biodiversity and safeguarding the functioning of aquatic ecosystems (Byers et al. 2002). In order to be successful, however, any management strategy must be based on a thorough knowledge of the dispersal, ecology and biology of introduced species and the effects that these exert on autochthonous communities. Relatively few ecological investigations on the goldfish have been carried out, despite its wide distribution; indeed, little information is available on goldfish populations in Italy (Lorenzoni et al. 2007, Pedicillo et al. 2007) and in Southern Europe in general (Tsoumani et al. 2006). The aim of our research was to collect information on the growth and reproductive biology of the goldfish, in order to investigate the causes of its rapid proliferation in Lake Trasimeno and to gather data on which to design a plan for the control of these unwanted populations.

### Study Area

Lake Trasimeno is a lake of tectonic origin situated within the Tiber River basin in central Italy (43°9'11" N and 12°15' E). It is the fourth largest lake in Italy (124.3 km<sup>2</sup>) and the largest in the Italian peninsula. Its shallowness (average depth: 4.72 m; max. depth: 6.3 m) makes Lake Trasimeno the largest laminar lake in Italy. Owing to the morphologic characteristics of Lake Trasimeno, the water temperature is almost the same as the air temperature, exceeding 30°C in the summer; thermal stratification is usually absent (Lorenzoni et al. 1993). Lake Trasimeno is classified as mesotrophic (Mearelli et al. 1990).

The fish fauna comprises 19 species and is dominated by cyprinids, including tench, carp and rudd (*Scardinius erythrophthalmus* L.); other common species are eel (*Anguilla anguilla* L.), perch (*Perca fluviatilis* L.) and sandsmelt (*Atherina boyeri* Risso). Today, fishing is still one of the main commercial activities of the local population and, despite

a decline in recent years, the number of professional fishermen is still higher than on any other inland lake in Italy. Profound changes have occurred in the composition of the fish community owing to the introduction of exotic species. The new species have adapted well, while the indigenous species have declined in abundance. The goldfish has been found in Lake Trasimeno since the 1990s. After a period of low population density, it gradually increased in number and is currently the dominant species caught by professional fishermen; meanwhile, catches of species of commercial interest, such as tench, carp, pike and eel, have declined (Lorenzoni et al. 2002).

### Material and Methods

Sampling was conducted monthly, from February 2003 to January 2004. Specimens were caught by means of electrofishing and nets at six sampling stations along the perimeter of the lake; two types of net were used: fyke-nets and gill-nets. The gill-nets were assembled from panels of different-sized mesh (22, 25, 28, 35, 40, 50 and 70 mm) to allow more efficient and representative sampling (Craig et al. 1986, Degerman et al. 1988). The panels, each of which was 1 m high and 50 m long, were positioned for one night near the bottom, perpendicular to the shore. The fyke-nets were positioned for one night in the vicinity of the gill-nets. Electrofishing was conducted monthly, except in April, when it was conducted weekly. Sampling was carried out from boats by means of 4.5 kW electric stunning devices; these devices supplied continuous pulsating current.

The standard length (SL) and total length (TL) of the specimens were measured to the nearest 1.0 mm; individual total weight (W) was recorded to the nearest 0.1 g (Anderson & Neumann 1996). The age of the fish was determined by means of the microscopic scalimetric method, and validated by means of length-frequency distribution (Bagenal 1978, Britton et al. 2004). The scales were removed from the left side of the fish, above the lateral line, near the dorsal fin (De Vries & Frie 1996) and stored in ethanol (33%) for reuse in back-calculation.

Specimens were assigned to age-classes on the basis of the number of winters that they had lived through before being caught. The date conventionally used as a cut-off between one age-class and the next was 31<sup>st</sup> December (De Vries & Frie 1996). As sampling was carried out in two different calendar years, the same specimen could be assigned to two different age-classes, depending on the period in which it was caught. For this reason, in the analyses of population

structure, the cohorts were identified according to the year of spawning of the individual specimens, rather than the age-class.

The TL-SL relationship ( $TL = a + b SL$ ) and TL-W relationship ( $W = aTL^b$ ) were calculated separately for the two sexes, by using a least-squares method (Ricker 1975). The relationships between the sexes were compared by analysis of covariance (Ancova).

In order to determine whether the growth of the goldfish is constant throughout the year, or whether, by contrast, it exhibits periods of stasis, the average length and weight by age were evaluated on a monthly basis in the 2+ and 3+ age-classes (Lorenzoni et al. 1993); comparison between the mean values calculated was made by means of the Tukey test. For the 1+ through 6+ age-classes, the TL and W mean values obtained for each age-class in the autumn and winter were also compared: comparisons were made by means of Student's *t*-test.

Back-calculated lengths were estimated for 51 females by examining four scales from each specimen and considering the mean of the observations. The scale radius ( $R_s$ ), from the centre of ossification to the edge of the scale in the caudal direction with an angle of  $45^\circ$ , and the radius of the age rings ( $R_t$ ), on the same axis, were measured for all scales ( $\pm 0.01$  mm) (Bagenal 1978) by means of an image-analysis system (IAS 2000). The relationship between the length of the specimen on capture (TL) and  $R_s$  was described by means of the Lee-Fraser regression  $TL = a + b R_s$ , which is widely used in studies on goldfish growth (Vetemaa et al. 2005). The result is linear and does not pass through the origin:  $TL = 4.322 + 26.859 R_s$  ( $R^2 = 0.885$ ). Back-calculated lengths were estimated by using the following formula:  $L_t - a = R_t / R_s (TL - a)$  (Bagenal 1978) in which  $a$  is the intercept with the axis of the abscissa of the previous regression and  $L_t$  is the TL of the fish at age  $t$ . Comparison of the sizes reached at the various ages between the two sexes was performed by means of a *t*-test. To determine whether Lee's phenomenon was operating, Anova with the *post hoc* Tukey test was used to compare the back-calculated lengths reached at the various ages of the specimens at age  $t$  with those of the samples of the older fish ( $t + i$ ). Theoretical growth in length was analysed by means of the von Bertalanffy (1938) equation:  $L_t = L_\infty (1 - \exp(-k(t - t_0)))$ , where  $L_t$  is the theoretical TL (in cm) at age  $t$ ,  $L_\infty$  the asymptotic length,  $k$  the coefficient of growth (in  $\text{years}^{-1}$ ), and  $t_0$  the theoretical age (in years) at length = 0 (Bagenal 1978). The analysis was conducted by

using only the TL values back-calculated from the last annulus. Back-calculated lengths for all annuli are commonly used in growth studies; however, in cases in which a Lee phenomenon occurs, the values of the parameters of the equation may be over- or underestimated (Vaughan & Burton 1994). To permit wider comparisons with the literature data, we also calculated the index of growth (in length) performance  $\phi'$ , which is derived by using von Bertalanffy parameters:  $\phi' = \log(k) + 2 \log(L_\infty)$  (Pauly & Munro 1984). The value of  $\phi'$  is influenced both by the intrinsic characteristics of the populations and by the environment in which they live; it therefore enables comparisons between different populations.

Sex was determined by microscopic examination of the gonads (Bagenal 1978) and gonads were weighed (Wg) to an accuracy of 0.1 g. The state of maturation of the ovaries was evaluated by means of Nikolsky's gonadal maturation scale (Nikolsky 1963).

The state of well-being of the fish in each lake was estimated by means of Fulton's condition factor:  $K_F = 100 W TL^{-3}$ , in which  $W$  is expressed in g and TL in cm (Bagenal 1978). The somatic condition factor ( $K_s$ ) was calculated by using the following formula  $K_s = 100 (W - W_g) TL^{-3}$ . Calculated in this way,  $K_F$  and  $K_s$  represent individual deviations from the hypothetically ideal fish displaying isometric growth, with and without gonad weight; this indices enable comparisons to be made between individuals, periods, age-classes and sexes (Weatherley 1972). As Clark (1928, in Froese 2006) pointed out, condition factors can only be compared directly either if  $b$  is not significantly different from 3 or the specimens to be compared are of similar length. To facilitate such comparisons, Le Cren (1951) introduced the relative condition factor ( $K'$ ), which compensates for changes in form or condition as length increases; in the equation  $K' = W (a TL^b)^{-1}$ ,  $W$  is expressed in g and TL in cm, and  $a$  and  $b$  are, respectively, the intercept on the y axis and the coefficient of the regression TL-W calculated on the whole sample. The somatic condition factor ( $K'_s$ ) was calculated by using the following formula:  $K'_s = (W - W_g) (a TL^b)^{-1}$ ; this index enables comparisons to be made between individuals, age-classes and sexes within the population (Weatherley 1972) even if the growth of the specimens proves to be allometric ( $b \neq 3$ ) (Froese 2006).

The ovaries of 92 females at stage V of maturation (Nikolsky 1963) were fixed in 10% buffered formalin immediately after removal. Three cross-sections of ovaries from each fish were weighed and microscopically examined, and the oocytes were



counted. Ten oocytes were selected for each female, and their diameter ( $\delta$ ) was measured by means of a computerised image-analysis system (IAS 2000) connected to the microscope. The relationship between SL and number of eggs ( $N_e = a SL^b$ ) was calculated by using a least-squares method (Ricker 1975). Relative fecundity (RF) was expressed as the number of eggs produced per unit of body weight:  $RF = N_e W^{-1}$ . In order to ascertain the existence of relationships between the size of the females and the parameters RF and  $\delta$ , Pearson's correlation analysis was carried out and the mean values of RF and  $\delta$  were calculated for size-classes of 50 mm SL.

## Results

The sample was composed of 3111 specimens. The TL, W, and age of the specimens analyzed varied between 4.30 and 40.60 cm, 1 and 1137 g, and 0.2 and 7.9 yr, respectively. Eight age-classes were found, with most specimens in the 2+ age-class. Sex was identified in 2057 specimens. Results showed that the population was composed mostly of females (males = 102, females = 1953). The females proved to be larger and older (mean  $\pm$  SE) (TL =  $23.24 \pm 0.18$  cm; W =  $354.03 \pm 6.69$  g; age =  $3.08 \pm 0.03$  yr) than the males (TL =  $15.69 \pm 0.63$  cm; W =  $96.73 \pm 14.15$  g; age =  $2.27 \pm 0.14$  yr); the differences between the sexes proved to be highly significant with regard to the three parameters analysed (Anova, TL:  $F = 298.40$ ,  $df = 2985$ ,  $p < 0.001$ ; W:  $F = 138.07$ ,  $df = 2985$ ,  $p < 0.001$ ; Age:  $F = 198.78$ ,  $df = 2985$ ,  $p < 0.001$ ). Females were grouped into 7 age-classes, while in the male subsample, 8 age-classes were found.

The SL-TL relationship calculated was:  $TL = 0.082 + 1.215 SL$  ( $R^2 = 0.99$ ,  $df = 3109$ ,  $p < 0.001$ ); covariance analysis revealed no difference between the sexes ( $F = 3.700$ ,  $df = 2053$ ,  $p = 0.054$ ). The TL-W relationship calculated was:  $W = 0.015 TL^{3.062}$  ( $R^2 = 0.99$ ,  $df = 1975$ ;  $p < 0.001$ ) for the overall sample;  $W = 0.015 TL^{3.051}$  ( $R^2 = 0.99$ ,  $df = 1543$ ;  $p < 0.001$ ) for the females, and  $W = 0.012 TL^{3.104}$  ( $R^2 = 0.98$ ,  $df = 95$ ,  $p < 0.001$ ) for the males; no significant difference emerged between the sexes (Ancova,  $F = 3.124$ ,  $df = 1638$ ,  $p = 0.077$ ). Because no difference emerged in the TL-SL and TL-W relationships, growth was analysed without distinction between the sexes.

## Growth

Analysis of the monthly trend in length and weight reveals that in the 2+ and 3+ age-classes growth does not take place at a uniform rate throughout the year (Fig. 1). Indeed, the 3+ age-class displayed

three periods of particularly rapid growth (January-February, June-August and September-October), for which the Tukey test revealed significant ( $p < 0.05$ ) differences in comparison with the mean values of W recorded in the following months; with regard to TL, these differences were significant only for January-February. Comparison of the mean lengths ( $\pm$  SE) of a given cohort in autumn with those reached by the same cohort in the following winter revealed significant increases:  $TL_{0+} = 8.58 \pm 0.31$  cm,  $TL_{1+} = 12.70 \pm 0.18$  ( $t = 12.39$ ,  $df = 109$ ,  $p < 0.001$ );  $TL_{1+} = 13.03 \pm 0.14$  cm,  $TL_{2+} = 16.57 \pm 0.47$  ( $t = 8.67$ ,  $df = 186$ ,  $p < 0.001$ );  $TL_{2+} = 21.87 \pm 0.14$  cm,  $TL_{3+} = 23.02 \pm 0.20$  ( $t = 4.71$ ,  $df = 226$ ,  $p < 0.001$ );  $TL_{3+} = 28.51 \pm 0.18$  cm,  $TL_{4+} = 29.48 \pm 0.34$ ; ( $t = 2.65$ ;  $df = 120$ ,  $p = 0.009$ );  $TL_{4+} = 33.25 \pm 0.16$  cm,  $TL_{5+} = 34.75 \pm 0.19$  ( $t = 6.04$ ,  $df = 102$ ,  $p < 0.001$ ) and  $TL_{5+} = 36.23 \pm 0.71$  cm,  $TL_{6+} = 35.98 \pm 0.84$  ( $t = 0.55$ ,  $df = 46$ ,  $p = 0.703$ ).

The back-calculated lengths at the various ages enabled us to evaluate the growth of the specimens (Table 1). The parameters ( $\pm$  SE) of the theoretical growth in length were  $L_{\infty} = 43.019 \pm 3.912$ ,  $k = 0.272 \pm 0.063$ ,  $t_0 = 0.162 \pm 0.086$ , the index of growth (in length) performance  $\phi'$  was 2.702.

Comparison of the back-calculated total lengths at the various ages between specimens of age ( $t$ ) and those of age  $t + i$  revealed that, in all age-classes, the mean values were higher in the older females ( $t + i$ ), except for the first annulus in the 4-year-old specimens (Table 1). On Anova, the differences between the mean values proved to be significant for the first annulus: the Tukey *post hoc* test revealed significant differences between the mean lengths of the 1-year-old specimens and those of the 4-year-old ( $p = 0.016$ ) and 5-year-old ( $p = 0.022$ ) specimens; this suggests the existence of an inverse Lee phenomenon.

## Condition

The mean value ( $\pm$  SE) of the Fulton condition factor ( $K_F$ ) in the overall sample was  $1.79 \pm 0.01$ , while that of the somatic condition factor ( $K_S$ ) was  $1.68 \pm 0.01$ . In females,  $K_F$  ( $1.78 \pm 0.01$ ) was higher than in males ( $1.70 \pm 0.02$ ); the difference proved to be highly significant on the  $t$ -test ( $t = 14.29$ ,  $df = 1853$ ,  $p = 0.001$ ).  $K_S$  was also higher in females ( $1.68 \pm 0.01$ ) than in males ( $1.67 \pm 0.02$ ) but in this case the difference was not significant ( $t = 0.182$ ,  $df = 1853$ ,  $p = 0.669$ ). The monthly trend in the sample of females shows that the best condition is reached at the end of the autumn (Fig. 2); indeed, the highest mean values of  $K_F$  and  $K_S$  were recorded in October ( $K_F$

$= 1.94 \pm 0.02$ ;  $K_s = 1.75 \pm 0.02$ ). From March to May the trends in  $K_F$  and  $K_s$  clearly diverged, because of the progressive emptying of the ovaries following reproduction; in May, when most of the eggs are laid, the somatic condition reached its annual minimum ( $K_s = 1.57 \pm 0.03$ ). The lowest mean value of  $K_F$  ( $1.66 \pm 0.02$ ) was recorded in February. From May onwards, the mean values of  $K_F$  and  $K_s$  again rose until November, both parameters displaying a fairly similar trend.

Further information on the trend in the condition of the goldfish and on its reproductive investment can be gleaned from the analysis of the relative condition factor ( $K'$ ) of Le Cren (1951) (Fig. 3). The mean relative condition is poor in the 1+ specimens, improves considerably in the subsequent age-classes and then steadily worsens, the lowest mean value being recorded among 6+ specimens. The differences among the mean values of  $K'$  and  $K_s$  proved to be highly significant on Anova ( $K'$ :  $F = 20.46$ ,  $df = 988$ ,  $p = 0.000$ ) ( $K_s$ :  $F = 24.43$ ,  $df = 988$ ,  $p = 0.000$ ); for both parameters, the *post hoc* Tukey test revealed significant ( $p < 0.05$ ) differences in comparison with the 1+ and 2+, 3+ and 4+, 4+ and 5+ age-classes. Comparison of the trends in the two parameters enables the differences in reproductive investment among the various age-classes to be evaluated. Indeed, the results reveal that the mass of the gonads is very low in the 1+ age-class, and thereafter steadily increases up to the 5+ age-class.

When the sample is broken down by season, the complete picture emerges (Fig. 4). In the 1+ age-class, the mean values of relative condition were particularly low in winter ( $K' = 0.89 \pm 0.01$ ;  $K_s = 0.88 \pm 0.01$ ) and spring ( $K' = 0.87 \pm 0.01$ ;  $K_s = 0.86 \pm 0.01$ ); in these seasons, the differences between the mean values of the two indexes were minimal and non-significant on Anova, indicating that gonadal development at this age is still very slight. In the subsequent seasons, the state of well-being of the specimens improved markedly; the differences between the mean values of  $K'$  and  $K_s$  always proved significant ( $p < 0.05$ ) on statistical testing, except for the summer values recorded in the 3+, 4+ and 5+ age-classes. In all the age-classes, except for 2+, the somatic condition reached its lowest mean value in spring, while the highest values were always reached in autumn. In winter, this parameter was always lower than in the previous autumn, indicating that survival is ensured in winter through the consumption of the reserves stored during the previous periods; the *post hoc* Tukey test

revealed significant differences between the autumn value of  $K_s$  in the 1+ class and the winter value in the 2+ class ( $p = 0.001$ ), between the autumn value in the 2+ class and the winter value in the 3+ class ( $p = 0.001$ ), and between the autumn value in the 3+ class and the winter value in the 4+ class ( $p = 0.001$ ).

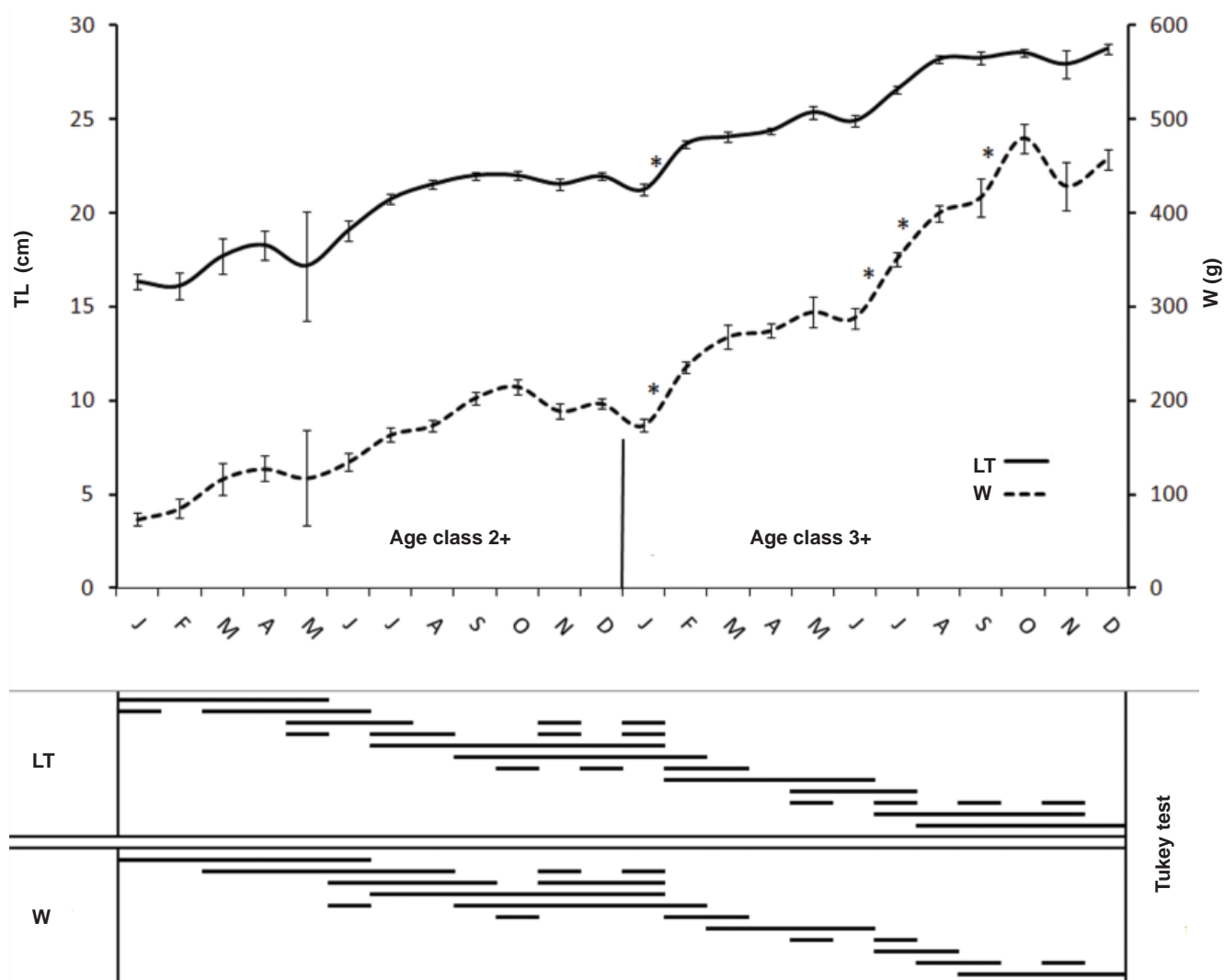
#### Age structure

Younger specimens were seen to be under-represented in the overall sample (spawned in 2003 = 8.40% of the total; spawned in 2002 = 18.60%). While the most numerous specimens were those of the cohort born in 2001 (26.74%), the previous age-classes back to the 1999 generation (18.40%) were also fairly abundant; older specimens became progressively rare (Fig. 5). The ratio between the sexes proved to be extremely unbalanced; 102 male specimens (2.60%) were observed, as against 1953 females (97.40%); the *sex ratio* was therefore 1 : 19.

#### Reproductive biology

Analysis of the stage of maturation of the gonads showed that all of the females carried stage-IV eggs at the end of the winter (January, February, IV = 100%) (Fig. 6). From March onwards, reproducing females were found (stage-V = 39.08%), their maximum percentage being reached in April (stage-V = 94.77%); the percentage was much lower in May (stage-V = 44.90%), when many had already laid their eggs (stage-VI = 55.10%). Reproducing females were, however, found up until June, though their frequency was extremely low (stage-V = 2.68%; stage-VI = 48.21%).

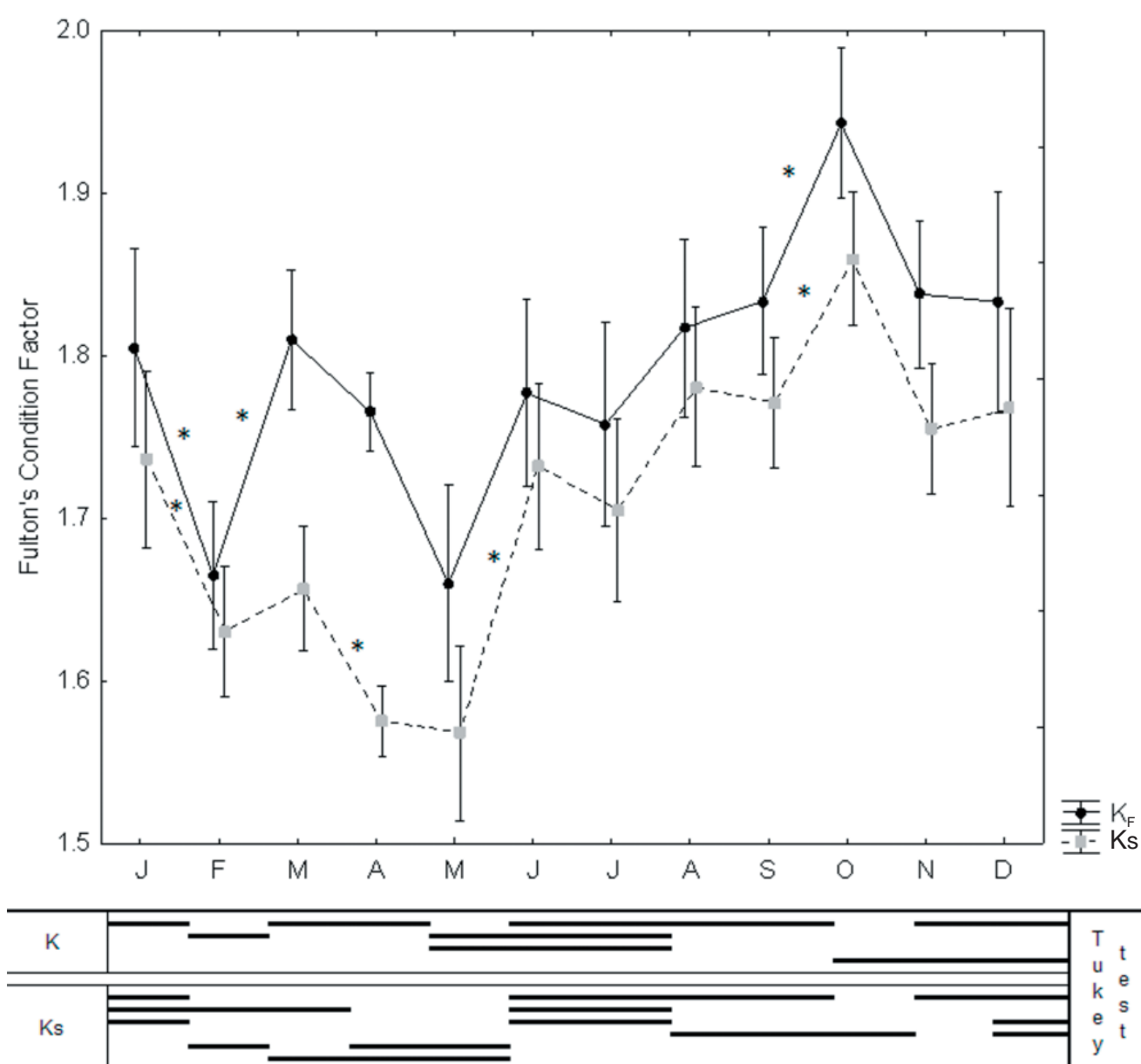
Most of the females with an SL of 130-139 mm had already reached sexual maturity (66.67% of the population sampled); the smallest sexually mature female was 100 mm in SL. Most of the females attained sexual maturity in the second year (85%), while in the third and subsequent years this percentage rose to 100%. However, some (7.55%) 1-year-old females were able to spawn. In males, sexual maturity is reached slightly earlier; in the 90-99 mm SL range, 76.92% of individuals were already sexually mature. Most of the males attained sexual maturity in the first year (60.61%), while in the second and subsequent years this percentage rose to 100%. The smallest sexually mature male was 90 mm in SL. The relationship between SL and the number of eggs was  $N_e = 0.0041 \text{ SL}^{4.368}$  ( $R^2 = 0.761$ ,  $df = 90$ ,  $p = 0.000$ ). Fecundity varied from 286 to 219104 eggs, averaging ( $\pm$  SE)  $46253 \pm 3921$  eggs. Mean RF was  $103 \pm 5$  eggs  $\text{g}^{-1}$ , varying from a minimum



**Fig. 1.** Monthly trend in growth in length and weight for the 2+ and 3+ age-classes. The lower panel shows the result of the Tukey test for homogenous groups; asterisks indicate statistically significant differences between successive months.

**Table 1.** Back-calculated total lengths in the female subsample of goldfish.

Age	No.	Length at age (mm):				
		I	II	III	IV	V
1	9	9.302				
2	16	10.528	15.714			
3	11	10.702	16.354	22.990		
4	11	11.862	17.982	24.764	29.910	
5	4	11.637	18.250	25.817	30.702	34.334
Mean		10.766	16.868	23.970	29.820	34.334
Confidence Limits at 95%		±0.494	±0.772	±1.257	±1.425	±2.681
Anova	df	46	38	23	13	
	F	3.28	2.54	2.12	1.88	
	p	0.019	0.072	0.142	0.202	



**Fig. 2.** Monthly trend in the Fulton Condition Factor in the female subsample of goldfish. The lower panel shows the result of the Tukey test for homogenous groups; asterisks indicate statistically significant differences between successive months.

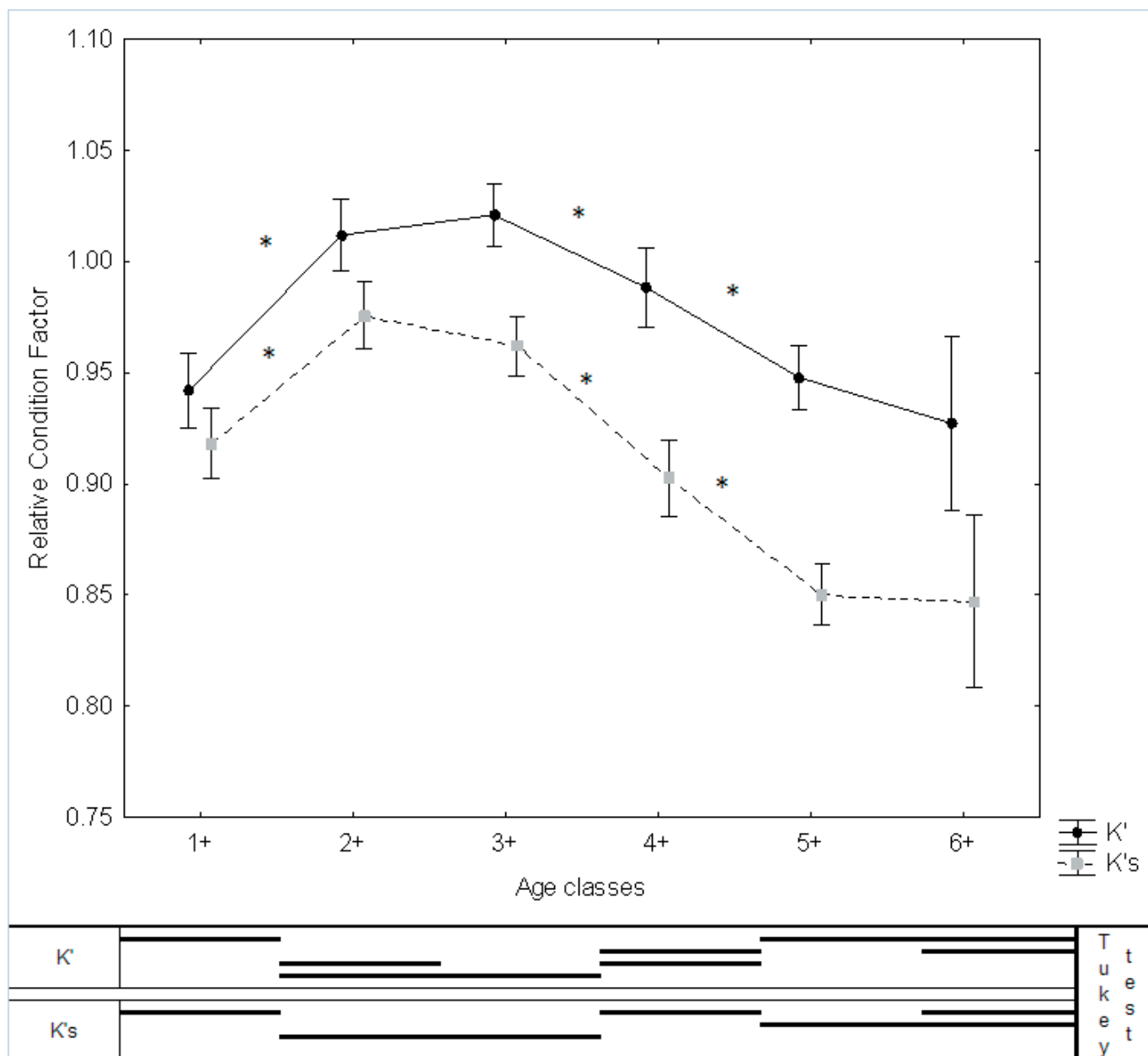
of 8.03 to a maximum of 276.73 eggs g<sup>-1</sup>. RF increased with the size of the specimens (Fig. 7), the differences being highly significant (Anova,  $F = 5.98$ ,  $df = 85$ ,  $p = 0.001$ ). This result was confirmed by correlation analysis: the number of eggs per unit of body weight correlated positively with SL ( $r = 0.468$ ,  $df = 89$ ,  $p = 0.001$ ). The diameter of mature eggs ( $\delta$ ) in the spawning season varied from 0.74 to 1.71 mm, with a mean of  $1.27 \pm 0.01$  mm. The value of  $\delta$  also displayed a steadily rising trend in parallel with the size of the specimens (Fig. 8) (mean value  $\pm$  SE: 100-150 mm SL =  $1.08 \pm 0.13$  mm; 150-200 mm SL =  $1.22 \pm 0.02$ ; 200-250 mm SL =  $1.28 \pm 0.01$  mm),

although in the larger females the mean value of  $\delta$  (300-350 mm SL =  $1.31 \pm 0.01$  mm) was slightly lower than in the preceding size-class (250-300 mm SL =  $1.33 \pm 0.01$  mm); the differences between the mean values of  $\delta$  proved to be highly significant (Anova:  $F = 63.30$ ,  $df = 85$ ,  $p = 0.000$ ). With regard to this parameter, correlation analysis indicated the existence of a positive correlation with SL ( $r = 0.605$ ,  $df = 89$ ,  $p = 0.000$ ).

## Discussion

An invasive species can be defined as any species that significantly adversely affects the long-term survival



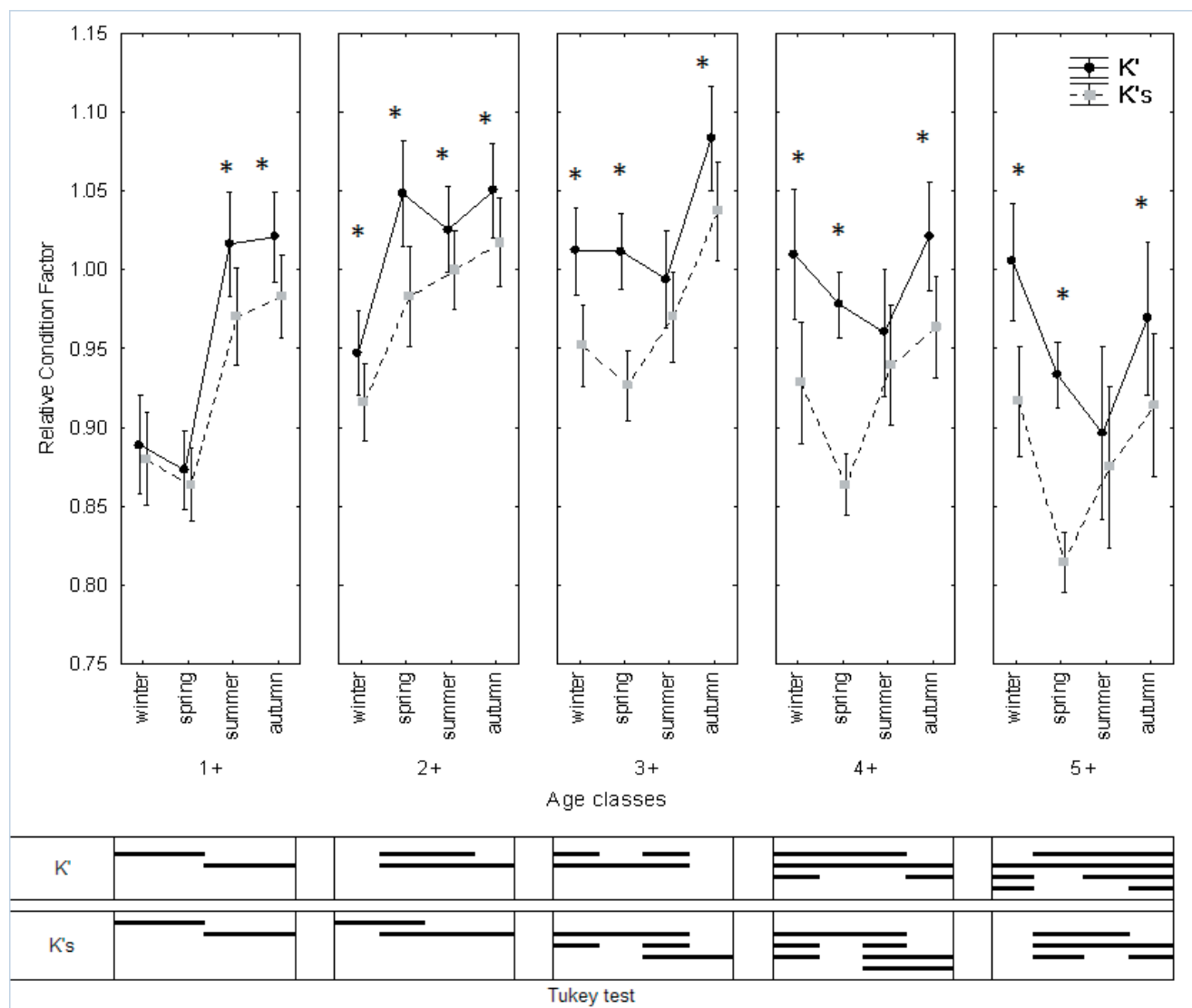


**Fig. 3.** Relative Condition Factor broken down by age-class of goldfish. The lower panel shows the result of the Tukey test for homogenous groups; asterisks indicate statistically significant differences between successive months.

or genetic variation of native species, or the integrity or sustainability of natural communities (Chadderton 2003, Copp et al. 2005). The biological features that determine the success of an invasive species are: rapid growth, high fecundity, a broad feeding spectrum and high tolerance of environmental conditions (Ruesink 2005). The main characteristics that determine the high invasive potential of the goldfish are, indeed, its great adaptability and ability to tolerate extreme environmental conditions (Abramenko et al. 1997). Another important factor in its success is its growth capability, which enables this species to rapidly reach a size that makes it safe

from predators. Its reproductive biology, precocious maturity, ability to reproduce by gynogenesis, and high fecundity, are also prerequisites to the invasive potential of this species (Peñáz et al. 1979, Vetemaa et al. 2005).

The goldfish has adapted very well to the environmental conditions of Lake Trasimeno; as a warm-water species, the goldfish is able to exploit the presence of shallow, eutrophic waters with dense vegetation. The population displays an age-structure in which the younger age-classes are under-represented in the overall sample. Longevity proved to be 8 years, which is in line with observations made



**Fig. 4.** Relative Condition Factor broken down by season and age-class of goldfish. Asterisks indicate statistically significant differences between  $K'$  and  $K's$  on the t-test. The lower panel shows the result of the Tukey test for homogenous groups.

in other environments in Italy (Pedicillo et al. 2007) and Europe (Muus & Dahlström 1967, Kuznetsov 2004, Vetemaa et al. 2005).

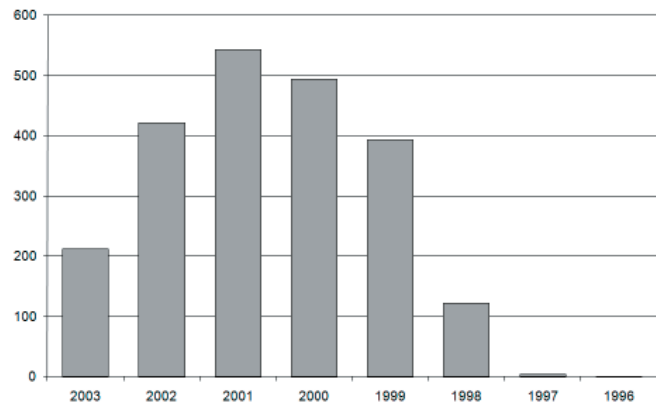
Our analyses did not reveal any sexual dimorphism in growth, though females have occasionally been found to grow more rapidly than males (Kuznetsov 2004). The regression coefficient  $b$  in the TL-W relationship calculated for the overall sample in Lake Trasimeno was 3.06, which indicates allometric growth. Analysis of the data available in the literature shows that the value of  $b$  in this species can vary from 2.72 to 3.24 (Alessio et al. 1996, Gomez et al. 1997, Pedicillo et al. 2007, Froese & Pauly 2008). In 12 Greek populations of *Carassius gibelio*, Tsoumani et al. (2006) recorded  $b$  values varying from 2.33 to

3.38, according to the trophic status of the lakes.

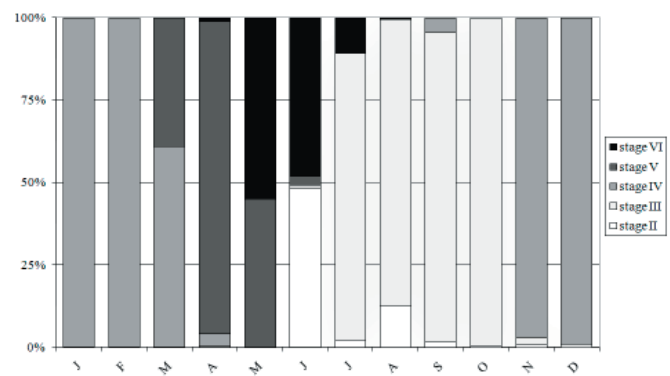
Back-calculation revealed that the formation of the annulus in the scales takes place around the month of April, in concomitance with the reproductive period. Growth is rapid: females slightly exceed 10 cm in total length at the end of their first year of life; at two years of age, their mean length is 16.87 cm, at three years, 23.97 cm, at four years, 29.82 cm and at five years, 33.50 cm. The parameters of the theoretical growth in length for the total sample in Lake Trasimeno were  $L_{\infty} = 43.019$  cm and  $k = 0.272 \text{ yr}^{-1}$ ;  $\phi'$  was 2.702. According to Taylor (1962), the von Bertalanffy equation provides a good description of the growth pattern if the maximum observed length is approximately 95% of the asymptotic

length; the maximum size of the specimens caught in Lake Trasimeno was 40.60 cm, which is equal to 94.40% of  $L_{\infty}$ . In Lake Corbara (Italy) the theoretical growth in length of the population is described by  $L_{\infty} = 49.18 \pm 1.15$  cm TL and  $k = 0.27 \pm 0.03$  yr<sup>-1</sup>, while  $\phi'$  is equal to 2.83 (Pedicillo et al. 2007). Kuznetsov (2004) and Vetemaa et al. (2005) have reported data on numerous European and Asiatic populations of goldfish; in comparison with these, the growth recorded in Lake Trasimeno generally proved to be more rapid.

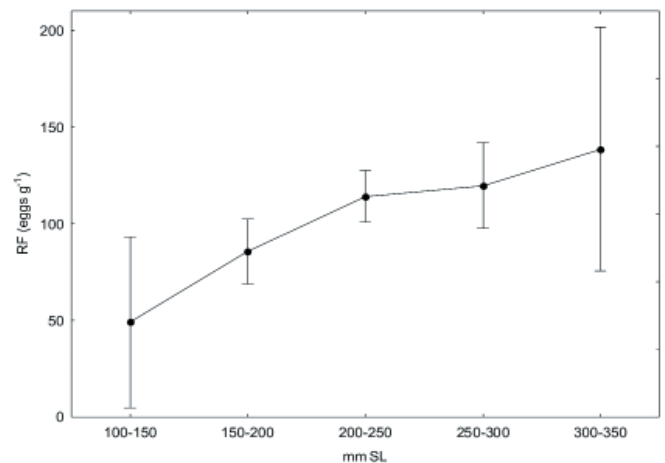
In Lake Trasimeno, growth does not take place in a continuous manner, but slows down in two periods of the year: late spring (May-June), which is the period of reproduction, and autumn-winter (November-January), when resources are less abundant. Comparison between the mean lengths of a given cohort in autumn and those reached by the same cohort in the following winter nevertheless reveals very significant increases. These increases are probably not due to true growth, but rather to differential mortality which penalises small specimens. Indeed, winter is a particularly critical period for many fish species on account of the low temperatures and reduced availability of food; mortality may therefore rise, especially among small and malnourished specimens as a result of intra- and inter-species competition (Buijse & Houthuijzen 1992, Ludsins & De Vries 1997, Lorenzoni et al. 2002). Back-calculation and analysis of the condition factor seemed to confirm the existence of these phenomena. Comparison of the back-calculated total lengths at the various ages between specimens of age  $n$  and those of age  $n + 1$  suggests the existence of an inverse Lee phenomenon. The origins of this may lie in the selectivity of the nets used in sampling, which may fail to catch the larger fish (Ricker 1975), or in the existence of very intense interactions among the younger specimens (0+ and 1+ age-classes) which favour those of larger size (Lorenzoni et al. 2002). This second hypothesis seems much more likely, in that the nets used in sampling, if they are selective, will lead to an overestimation of the larger specimens in the sample (Cowx & Lamarque 1990). Predation is one of the main causes of mortality in many fish populations, and the goldfish appears to be particularly vulnerable to this (Bronmark et al. 1995). The direct effects of predation may include a reduction in population density and, if the predation is size-dependent, an alteration in population structure, thus causing an inverse Lee phenomenon (Holopainen et al. 1991, Tonn et al. 1991).



**Fig. 5.** Age structure of the overall sample of goldfish.

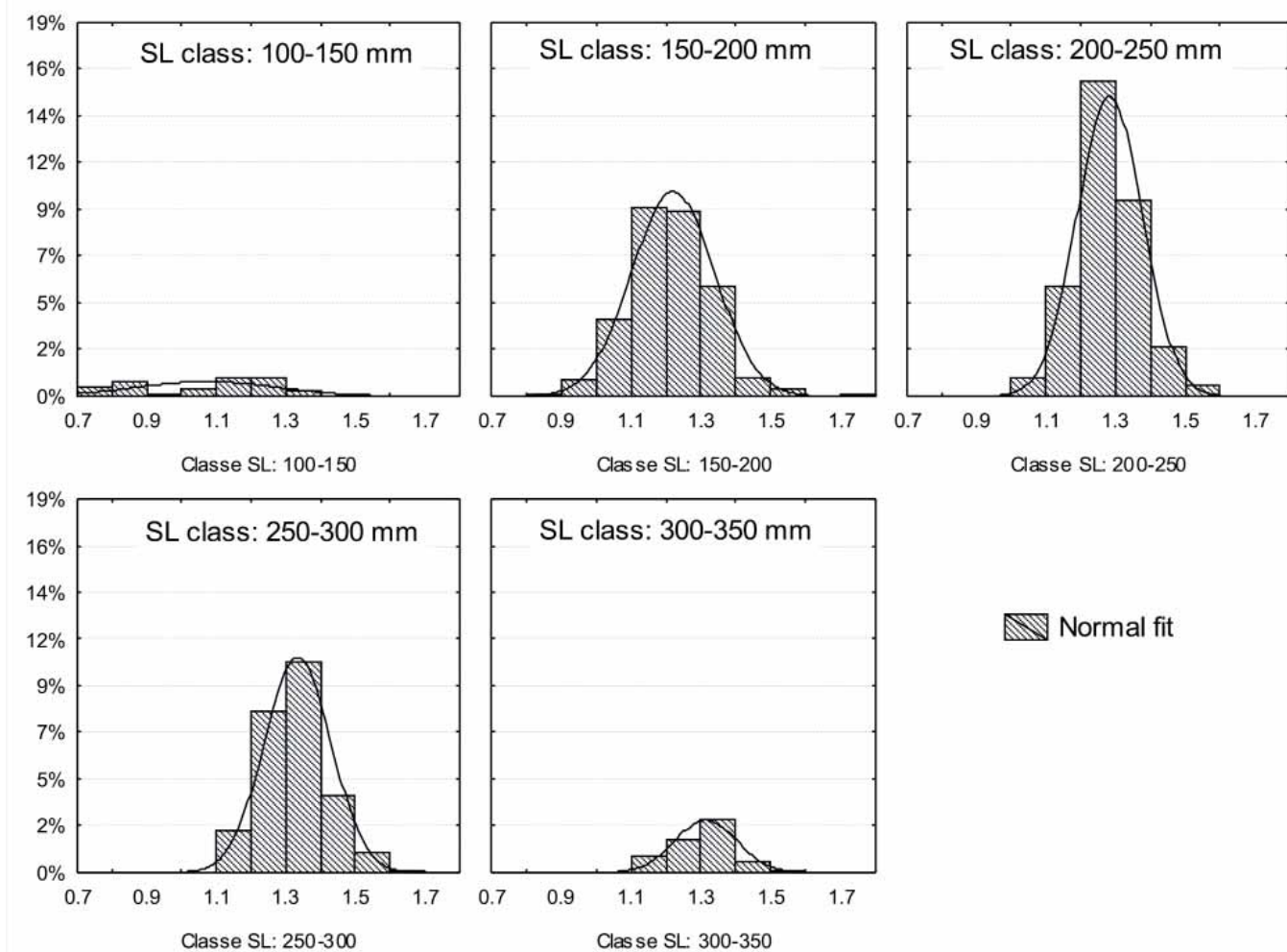


**Fig. 6.** Monthly trend in the percentages of stages of maturation of ovaries in the female subsample of goldfish.



**Fig. 7.** Relative fecundity (RF) broken down by size-classes of 50 mm SL in the female subsample of goldfish.

The condition of the goldfish population in Lake Trasimeno appears to be good, though not as good as that of other populations in nearby waterways. The mean value of the Fulton condition factor ( $K_F$ ) is fairly high (1.79); this is probably due to the



**Fig. 8.** Histograms of relative frequency of egg diameters ( $\delta$ ) broken down by size-classes of 50 mm SL in the female subsample of goldfish.

morphological features of the species, which tends to be stocky rather than elongated. In the Corbara Reservoir, the mean value of  $K_F$  ( $\pm$  SE) has been reported to be  $1.88 \pm 0.01$  (Pedicillo et al. 2007), while in the entire Tiber River basin it is  $1.82 \pm 0.01$  (Lorenzoni, unpublished data). Analysis of the seasonal trend in the condition factor ( $K_F$ ) of the goldfish in Lake Trasimeno reveals that autumn is the most favourable season, and that the population displays its worst condition in winter. In summer, the greater availability of food and the favourable weather conditions permit rapid growth and the accumulation of reserves of fat; this justifies the high value of  $K_F$  recorded in autumn. By contrast, in winter the adverse weather conditions, the scarcity of food and especially the slowing down of the fish's metabolism reduce the growth rate of the goldfish, thus justifying the low  $K_F$  value recorded in that season. In spring, the condition of the specimens

is also influenced by reproduction, in which the females invest large amounts of resources, so much so that this is the very period in which the somatic condition ( $K_S$ ) reaches its lowest value. On analysing the variations in the relative condition factor in the different age-classes, it can be seen that, in the Lake Trasimeno population, the 1+ specimens present a rather poor condition, while the central age-classes (2+ and 3+) display uniform and fairly high mean values of  $K_F$ ; in the older age-classes (4+ and above) the state of nutrition progressively deteriorates. This trend again points to the possible existence of environmental factors that particularly penalise the younger specimens, thereby preventing them from attaining a satisfactory condition. It seems likely that the current population levels engender particularly intense intra-species competition which has a particularly negative impact on the younger individuals. By contrast, in the older age-classes, the

poor somatic condition observed may be explained by the increase in reproductive investment that takes place during ageing. This appears to be fully confirmed even when the seasonal trend in the relative condition factor is broken down according to age-class; in this case, too, specimens of the 1+ class display particularly low mean values of the relative condition factor (both general and somatic) in winter. The same analysis also reveals that the older age-classes have a very poor somatic condition in spring, and that the trend in this factor appears to be related to the increase in reproductive effort and ageing.

Analysis of the reproductive biology of the goldfish population in Lake Trasimeno revealed that sexual maturity in females is reached after the second winter of life (2+ age-class), when over 85% display mature gonads; however, a small percentage (7.55%) of females are already able to reproduce at the age of one year, while from the third winter onwards the entire female population is involved in egg laying. The most precocious females are able to reproduce at an SL of slightly more than 10 cm. In males, sexual maturity is reached even earlier: most of the males attained sexual maturity in the first year (60.61%), while in the second and subsequent years this percentage rose to 100%. The smallest sexually mature male was 90 mm SL. In Lake Massaciuccoli, in Tuscany, goldfish are reported to reproduce for the first time at the age of one or two years (Sani et al. 1999).

Reproductive investment is very high among the females, exceeding 50% of the entire body weight at its peak: the mean value of GSI is  $6.25 \pm 0.19$  (Lorenzoni et al. 2007). In Lake Trasimeno this reproductive investment is not homogeneous throughout the age-classes; fecundity is low in 1+ females, but steadily rises in the subsequent age-classes. This contrasts with what has been observed in Lake Massaciuccoli, where age does not seem to influence fertility (Sani et al. 1999). In Lake Trasimeno the relationship between SL and the number of eggs was  $N_e = 0.0041 \text{ SL}^{4.368}$  and fecundity varied from 286 to 219104 eggs, averaging  $46253 \pm 3921$ ; the figures generally reported in the literature range from 160000 to 380000 eggs (Muus & Dahlström 1967), which coincides fairly well with our observations. In Lake Massaciuccoli (Sani et al. 1999) the absolute fecundity of female goldfish displays a mean value of 63211 eggs (min = 23612, max = 79804), while in Corbara Reservoir mean absolute fecundity is 62515 eggs (min = 17600, max = 135800) (Pedicillo et al. 2007). In Lake Trasimeno, mean RF was  $103 \pm 5 \text{ eggs g}^{-1}$ , while in

Corbara Reservoir relative fecundity varies from 18.38 to 125.60 (mean =  $72.31 \pm 4.41$ )  $\text{eggs g}^{-1}$  (Pedicillo et al. 2007). In Lake Trasimeno the diameter of mature eggs in the spawning season ranged from a minimum of 0.74 to a maximum of 1.71 mm, with a  $1.27 \pm 0.01$  mm mean; this value is slightly below that reported by Bruno & Maugeri (1992) as the typical range in Italian populations (1.4–1.7 mm); in Lake Massaciuccoli, mean  $\delta$  is 1.17 mm (min = 1.06, max = 1.37 mm). In Lake Trasimeno the size-related increase in reproductive investment is also confirmed with regard to RF; the fact that a similar relationship is observed with regard to  $\delta$  indicates that not only the quantity but also the quality of the eggs produced increases with age.

The reproductive period of the population covers a broad time-span, which runs from March to June. The goldfish does not lay all its eggs at the same time, but rather at intervals (Muus & Dahlström 1967, Gandolfi et al. 1991). The water temperature at which reproduction takes place in Lake Trasimeno is about 13°C; this is lower than the temperature (20°C) reported by Gandolfi et al. (1991), but in line with the observations made by Kuznetsov (2004) with regard to the Volga basin (12–14°C). Similar findings to those recorded in Lake Trasimeno have been reported in Lake Massaciuccoli (Sani et al. 1999), in which the reproductive period runs from May to June, though some individuals occasionally reproduce in March. In Lake Pamvotis (Greece) reproduction occurs during March and April, at a temperature of 12°C (Paschos et al. 2004).

One of the main biological traits responsible for the success of the goldfish as an invader is its ability to reproduce by means of gynogenesis (Flajšhans et al. 2008). Indeed, the populations introduced into Europe are often made up almost exclusively of triploid females which exhibit gynogenetic reproduction (Vetemaa et al. 2005). The *sex ratio* observed in Lake Trasimeno is extremely unbalanced, with males accounting for only 5.26% of the population. Similar deviations in the *sex ratio* have been observed in many European populations (Abramenko et al. 1997, Sani et al. 1999, Kuznetsov 2004, Vetemaa et al. 2005, Pedicillo et al. 2007). The development of gynogenetic eggs is initiated by the activation of mitotic division on the part of heterologous spermatozoa (Muus & Dahlström 1967, Peñáz et al. 1979, Zou et al. 2001). In Lake Trasimeno, sampling netted numerous specimens of exclusively male carp and rudd, intermingled with reproducing goldfish. In Lake Pamvotis (Greece) the small percentage (2.3%) of



males has been quoted as evidence that, in addition to gynogenetic reproduction, gonochoristic reproduction is an alternative option (Paschos et al. 2004, Flajšhans et al. 2008). In Lake Trasimeno this would seem to be confirmed by the fact that, at the time of reproduction, the males were able to render milt. Indeed, it is known that males sometimes have testicles that are not fully developed and cannot therefore take part in reproduction (Vetemaa et al. 2005).

Localized infestations of invasive species may be eradicated through early detection and rapid response efforts. Eradication of widespread invasive species, however, may not be feasible. Widespread invasive species are subject to control and management efforts designed to restore an altered system to its previous state or lessen the impact of invasive populations. The control of an invasive species may be defined as a management action aimed at reducing the density of pest species: it is essentially a sustained-yield operation in localities where landings of unwanted species were formerly absent (Elvira 2000). The adoption of a particular strategy to control the population of invasive species depends both on the assessment of its costs/benefits and on its potential for success (Myers et al. 2000). Generalization is difficult, as the choices vary according to several

aspects, which also regard the economic and social context. Nevertheless, the quantification of age, growth and reproductive biology is a fundamental component in prioritizing control and management operations (Kwak et al. 2006). Important indications will also emerge from data on catches of goldfish and on selectivity of the various types of sampling gear; although these data are still being processed, it is already possible to make a few preliminary considerations. The existence of natural mortality resulting from intra-species competition, which chiefly involves the younger age-classes, suggests that intervention should not be aimed at containing this part of the population, in that the existence of density-dependent mechanisms could easily offset the losses caused by culling. The results of the analysis of the reproductive biology of the Lake Trasimeno population suggest that intervention should rather be aimed at those age-classes that make the greatest contribution to natural recruitment. Containment initiatives may also be facilitated by the tendency of sexually mature goldfish to congregate in restricted areas (Paschos et al. 2004) and by the particular efficacy of electrofishing in capturing this species in Lake Trasimeno (Lorenzoni et al. 2007).

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