

## Variation in clones of the sperm-dependent parthenogenetic *Carassius gibelio* (Bloch) in Lake Pamvotis (north-west Greece)

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(Received 5 June 2007, Accepted 24 September 2007)

Variation in three different clones of the invasive sperm-dependent, cyprinid fish *Carassius gibelio* were examined in Lake Pamvotis (north-west Greece). Differences between the clones were found in their proportion in the population, in their age structure, in the time of arrival to their spawning grounds and in the coefficients of the von Bertalanffy growth equation.

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Key words: *Carassius gibelio*; growth rate; intraclonal variation; sperm-dependent parthenogenesis.

The Prussian carp *Carassius gibelio* (Bloch, 1782) is an invasive freshwater, benthipelagic, omnivorous species which is widely distributed and flourishing from Europe, including Greece (Kottelat, 1997), to the Japanese Islands. Its populations consist mainly of triploid females (150 chromosomes) which reproduce by sperm-dependent parthenogenesis (Zhou *et al.*, 2000). Many asexual populations are composed of numerous ecologically different clones that arose independently from diversified sexual ancestors and flourish in different environments (Vrijenhoek, 1998). Clones usually have high levels of ploidy and may differ in their morphology, physiology, life-history traits and behaviour (Schultz & Fielding, 1989; Lima & Vrijenhoek, 1996). Such diverse clones provide natural experiments that allow biologists to study interactions between fixed genotypes and environments.

Analysis of genetic heterogeneity among gynogenetic populations in China (Zhou *et al.*, 2000), Italy, Germany and Britain (Hanfling *et al.*, 2005) and Greece (Moutsaki *et al.*, 2006) based on genetic analysis has revealed different clones among each population. The Chinese clones differ in body type, growth, spawning time and other characters (Zhu & Jiang, 1993), while the Greek

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clones in Lake Pamvotis differ in their morphology (I. D. Leonardos, P. Moutsaki, A. Triantafyllidis, R. Liasko, E. Kappas & I. J. Abatzopoulos, unpubl. data). Given that Prussian carp is a unique species with coexistence of sexual (Zhaoting & Junbao, 1990) and asexual reproduction (Zhou *et al.*, 2000), multiple clones (Zhou *et al.*, 2000; Hanfling *et al.*, 2005; Moutsaki *et al.*, 2006) and levels of ploidy (Toth *et al.*, 2005; Liasko *et al.*, 2007), the knowledge of its growth patterns is of great importance.

In order to compare the growth of the three clones in the small shallow Lake Pamvotis, north-west Greece (39°40' N; 20°53' E), 464 fish were collected during the period of 2004–2006 from their spawning grounds using a portable electro-fishing device IG200/2 (Hans Grassl, 250W, 25A, 5-10KW/Imp; Hans Grassl GmbH, Schönau am Königssee, Germany). Spawning grounds are in the littoral zone in places with dense submerged vegetation. The fish were returned to the lake after examination.

Genetic analysis of 143 out of the 464 specimens revealed the presence of three clones in the Lake Pamvotis (Moutsaki *et al.*, 2006). Based on a multivariate analysis of 39 morphometric and five meristic features, the discriminant function analysis (DFA) revealed that the three clones are 100% morphologically distinguishable (I. D. Leonardos, P. Moutsaki, A. Triantafyllidis, R. Liasko, E. Kappas & T. J. Abatzopoulos, unpubl. data). Using one of the main features of DFA, which is the classification of an observation or several observations into already known groups (Hardle & Simar, 2003), the same truss protocol was used for the entire population. Using the already known clones (from the original 143 specimens) as a selection variable, the DFA classified the 464 specimens with an accuracy of 96.5%. The multivariate analysis, that was used to identify morphological differences between the clones of the 464 specimens, revealed the same DFAs with those of the 143 specimens.

For each fish standard length ( $L_S$ ) ( $\pm 0.1$  mm) and total mass ( $M_T$ ) ( $\pm 0.1$  g) were measured in order to calculate the Fulton's condition factor: ( $K$ ) from  $K = 100M_T L_S^{-3}$  (Bagenal & Tesch, 1978), which is suitable for comparing different specimens of the same species and indicates differences related to clones. Statistically significant differences were found in  $K$  in relation to the clones (mean  $\pm$  s.e. first clone:  $K = 3.09 \pm 0.07$ , second clone:  $K = 2.71 \pm 0.06$  and third clone:  $K = 2.60 \pm 0.14$ ; ANOVA,  $F_{2,467}$ ,  $P < 0.001$ ). The  $L_S$  frequency distribution of 464 fish of the three clones is shown in Fig. 1.

All the specimens were mature and were studied prior to the reproductive period. Sex was determined from the examination of the gonads by slight pressure in the abdominal. The Prussian carp samples caught in Lake Pamvotis consisted mainly of females. Males were found in a very small portion (<1%). Particularly remarkable was the absence of males in the third clone. The first clone seemed to be prevalent (58.20%), followed by the second clone (32.11%) and the third clone at the lowest level (9.70%) ( $\chi^2$ , d.f. = 2 at  $P < 0.001$ ).

Age was determined in 464 specimens. Scales were removed from the left side of the fish, between the posterior end of the pectoral fin and the anterior end of the dorsal fin. Annual rings were recognized by standard criteria (Bagenal & Tesch, 1978). Scale reading indicated six age classes for each of the three clones. The first clone consisted mainly of 1 and 2 year-old specimens, the second clone of 1, 2 and 4 year-old specimens and the third clone mainly of

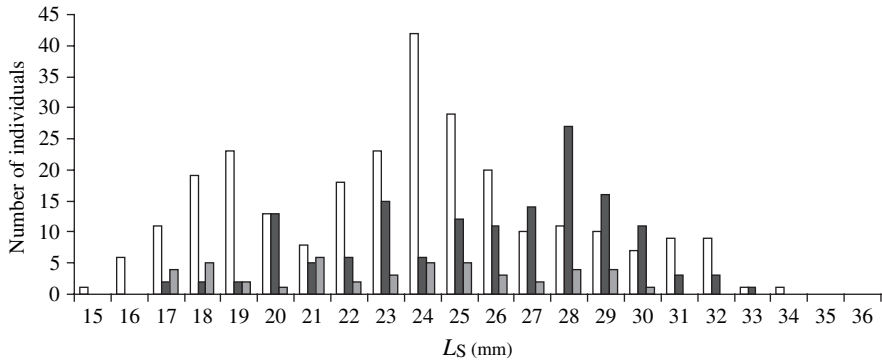


FIG. 1. Standard length ( $L_S$ ) frequency distribution of *Carassius gibelio* clones (□, 1; ■, 2; ▒, 3) caught in Lake Pamvotis.

1 year-old specimens. The age structure of the different clone populations were significantly different ( $\chi^2$ , d.f. = 12,  $P < 0.001$ ).

The time of arrival at the spawning grounds peaked in March, April and May for the first, second and third clone, respectively ( $\chi^2$ , d.f. = 6,  $P < 0.001$ ). Contrary to the Russian population (Kuzmenko, 2002), there was no statistically significant correlation between age of clones and time of arrival at their spawning grounds ( $P > 0.05$ ).

The relationship between  $L_S$  and total scale radius ( $R$ ) was examined for all individuals and for each clone separately testing for differences in slopes and intercepts using ANCOVA (Zar, 1999). The  $L_S$  and  $R$  regressions were tested in relation to the clone (Table I); the slopes differed significantly ( $F_{2,461}$ ,  $P < 0.001$ ). The specimens of the first clone grow faster than those of the other clones (clone 1 and clone 2:  $F_{1,415}$ ,  $P = 0.01$ ), (clone 1 and clone 3:  $F_{1,318}$ ,  $P = 0.05$ ). The specimens that belonged to clone 3 grew marginally faster than the specimens of clone 2, but no statistically significant differences were found ( $F_{1,193}$ ,  $P = 0.05$ ).

Backcalculations of  $L_S$  at age were estimated from a modified version of the direct proportion formula (Lee, 1920):  $L_{Sn} - c = R_n R^{-1} (L_S - c)$ , where  $L_{Sn}$  is the standard length when the annulus  $n$  was formed,  $L_S$  the standard length at time of capture,  $c$  is the intercept on the length axis from linear regression of  $L_S$  on scale radius,  $R_n$  the distance from scale focus to annulus  $n$  and  $R$  the scale radius. The specimens of the first clone reached 41.49% of their maximum adult size during the first year and 23.66% during the second year. The growth of the specimens of the second clone was higher during the first year, reaching

TABLE I. The coefficient values ( $a$  and  $b \pm 95\%$  CI) of the relationship between standard length ( $L_S$ ) and scale radius ( $R$ ) for the three clones in Lake Pamvotis, where  $L_S = a + bR$

Clone	$a$	$b$	95% CI of $b$	$n$	$r^2$	$P$ -value
1	4.58	0.19	0.17–0.20	270	0.76	<0.001
2	12.07	0.11	0.09–0.13	149	0.55	<0.001
3	9.36	0.13	0.11–0.15	45	0.70	<0.001

TABLE II. The von Bertalanffy growth coefficients of the three clones

Clone	$L_{S\infty}$ (95% CI) (mm)	$k$ (95% CI)	$t_0$ (95% CI) (years)	$r^2$
1	31.58 (30.13–33.03)	0.52 (0.45–0.59)	-0.02 (-0.11 to 0.83)	0.87
2	28.64 (27.69–29.59)	0.66 (0.54–0.79)	-0.42 (-0.63 to -0.21)	0.81
3	26.54 (25.27–27.81)	0.87 (0.61–1.13)	0.05 (-0.19 to 0.29)	0.88

at 55.24% of its maximum adult size, followed by a sharp decline during the next years. The specimens of the third clone reached 52.09% of their maximum adult size during the first year and 23.57% during the second year.

The von Bertalanffy growth curve (von Bertalanffy, 1938) was fitted to data of  $L_S$  at age by means of the Marquardt's algorithm for a non-linear least squares parameter estimation. The coefficients of the von Bertalanffy growth equation are shown in Table II. As revealed by the von Bertalanffy growth curves, the three clones differed in relation to their asymptotic  $L_S$  ( $L_{S\infty}$ ) and were partially distinguished by their growth coefficient ( $k$ ). In particular, the  $L_{S\infty}$  values are highest for the first clone, medium for the second and lowest for the third clone. The value of  $k$  also varied and was highest for clone 3. Values for  $L_{S\infty}$  for the three clones agreed well with the observed  $L_S$ . The largest specimens collected during this study were 319 mm for the first clone, 298 mm for the second clone and 274 mm for the third clone.

In its original environment, *i.e.* the rivers and lakes of Asia (Penaz & Dulmaa, 1987) many populations of *C. gibelio* were found consisting of specimens >8 years old and with different von Bertalanffy growth equation coefficients. As *C. gibelio* was introduced in Greek fresh waters, growth, values of  $L_{S\infty}$  and  $k$  vary significantly from those in its natural habitat (Specziar *et al.*, 1997; Vetemaa *et al.*, 2005). The high growth rate during the first years of its life, combined with other biological characters such as sperm-dependent parthenogenesis, explains the success of the Prussian carp population in the lake since the early 1980s when it was first introduced. Since the early 1980s, Prussian carp has gradually increased in numbers and is currently the dominant species in the lake (Leonardos *et al.*, 2007).

It is clear therefore that the three clones not only differ in heterogeneity (Moutsaki *et al.*, 2006) but also exhibit different growth patterns. Taking into account that as the clones reproduce asexually (develop from unfertilized eggs) and genetical recombination is absent any difference between the clones is attributed exclusively to their genetical composition (Vrijenhoek, 1998) similarly to the Chinese population (Zhu & Jiang, 1993). So contrary to the belief that individual clones are evolutionary dead ends as they lack the ability to respond genetically to changes of their physical and biotic environment (Vrijenhoek, 1998), the fact that the population of *C. gibelio* in Lake Pamvotis consists of multiple clones with various biological characteristics and life traits, allows them to flourish under a variety of ecological conditions.

This research was funded by the programme 'Pythagoras I' of the Operational Programme for Education and Initial Vocational Training of the Hellenic Ministry of Education under the third Community Support Framework and the European Social Fund.

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