Vestnik zoologii, **50**(5): 423–428, 2016
DOI 10.1515/vzoo-2016-0049

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UDC 597.551.2(285.33: 477)

BREEDING POTENTIAL OF ADVENTITIOUS SPECIES OF *CARASSIUS AURATUS* AND *CARASSIUS GIBELIO* (CYPRINIFORMES, CYPRINIDAE) IN WATER BODIES OF UKRAINE

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Breeding Potential of Adventitious Species of *Carassius auratus* and *Carassius gibelio* (Cypriniformes, Cyprinidae) in Water Bodies of Ukraine. Kokodiy, S. V. — The following proofs of Goldfish more powerful reproductive potential are given: high individual and relative fecundity, adequate fractional spawning process resulting in numerous offspring, represented by different generations of hatchlings, little number of individuals not participating in reproduction, prevailing number of fish eggs in females' ovaries, larger fraction of impregnated roe. All the above mentioned factors allow claiming that the Prussian carp is being replaced due to its low replacement ability. Perhaps for that reason *Carassius gibelio* (Bloch, 1782) choose small isolated water bodies, where it makes small populations, backed up by successful spawning with a single male of crucian carp, *Carassius carassius* (Linnaeus, 1758).
Key words: fecundity, individual fecundity, relative fecundity, breeding potential, Goldfish, *C. auratus*, Prussian carp, *C. gibelio*, crucian carp, *C. carassius*.

The problem of cross-species relations of invasive species in new environment has slipped from the academic community's attention because more attention was devoted to the research of trivial invasions, hybridization of adventive species with native species and finding out the reasons of the latter's extinction. Contemporary research proved that a peculiar struggle for existence took place not only between the invadors and native species, but between the adventitious species too. Such complicated cross-species relations occur between the representatives of the genus *Carassius*.

Application of genetic methods for identification of morphologically indistinguishable representatives of the Prussian carps, *Carassius auratus* (Linnaeus, 1758) s. l., gave an opportunity for studying the Prussian carps' genetic structure. It became known that not one species, as it was considered earlier, had penetrated the Ukrainian water bodies but a few species with different ploidy: diploid amphimictic Goldfish, *C. auratus* (Linnaeus, 1758), and triploid apomictic species the Prussian carp, *Carassius gibelio* (Bloch, 1782), which was represented by a few clonal forms (Mezhzherin, Lisetzkiy, 2004 a, b; Mezhzherin, Kokodiy, 2010). It turned out that there was a distinct tendency for substitution not only of native species of the crucian carp, *C. carasius*, but also adventitious species of the Prussian carp, *C. gibelio,* for Goldfish, *C. auratus*, in common settlements. (Mezhzherin et al., 2015). That is the formation of homogenous settlements with individuals of single amphimictic species *C. auratus* takes place. Such situation is a unique and unexplored one, since similar cases are very rare in the wild.

What was the reason for such relations between the two adventitious species is not reliably known. In case with the crucian carp, *C. carasius,* the cross-species hybridization occurred, which resulted in genetic inception of native species, but in case with the clonal form of the Prussian carp, *C. gibelio,* the other type of reproductive relations can be observed. The settlements of apomictic Prussian carp, *C. gibelio,* are represented by females reproducing by means of gynogenesis, using male sperm cells of other carp species and Goldfish, *C. auratus,* in particular. Theoretically the mixed population of two adventitious species must exist in balanced state, because the Prussian carp females in some way "parasitize" the Goldfish, *C. auratus,* males. However, the preliminary analysis proves (Mezhzherin et al., 2007), that *C. auratus* and *C. gibelio* are not liable to coexistence with each other. There is a tendency everywhere to formation of homogeneous settlements consisting of the representatives of the first species. Hypothetically the reason for such substitution is more powerful breeding potential of Goldfish, *C. auratus*, which significantly exceeds the one of Prussian carp, *C. gibelio*. This research aims to study the reasons and peculiarities of such profound changes.

Material and methods

The research basis was the series of the crucian carp selections, consisting of 447 adults and 134 hatchlings, collected in 2013–2014 in Spring–Autumn periods from two testing water bodies related to the Dnepr river's catchment area. The species identity was determined by allozyme markers (Mezhzherin, Lisetzkiy, 2004 a, b). The following was analyzed in the muscles: the serum glutamate-oxaloacetate-transaminase, different forms of which are coded by corresponding locus Aat-1, Aat-2; nonspecific esterases (Es-1, Es-2A, Es-2B); structural muscle proteins (variation Pt-2 was studied). The transferrins (Tf) were studied in the blood. The age was determined in a standard way by the age rings on the scale (Pravdin, 1966), the sex was determined by the presence of male or female reproductive products, sexual maturity by the gonads' development. Crucian carps' fecundity was studied by calculating the fish eggs in 1 gram. The crucian carps' bodies were measured in a standard way (Pravdin, 1966). The collected data was processed by the programs Excel and Past.

Results and discussion

For studying the individual and relative fecundity and controlling the progeny of the two species the two similar water bodies were chosen. In one of them the crucian carps were represented only by digeneous diploid individuals of Goldfish, *C. auratus*, in the other one by polyploid unisexual individuals of the Prussian carp, *C. gibelio*. There was no difference in the rate of growth, body weight and Fulton nutrition status in age groups from the examining water bodies.

The species identification was implemented via gene-marking. It ought to be remarked that Goldfish is easily detected via polymorphous electrophoretic spectra, which reflect high degree of allozyme mutation of these species' populations. The Prussian carp individuals were identified by the range of allozyme spectra, a part of which is coded by constant heterozygotes with unique alleles unusual for *C. auratus*, in particular Aat-2^{110}, Gpi-1^{90}. The solitary crucian carps, *C. carasius,* were found in the water body with Prussian carps, apparently the crucian carps were spermium donors for the genetic species. The crucian carps, *C. carasius*, are easily detected by the morphological characters. In the landings it was represented by juvenile individuals.

It was proved after reproductive products' analysis of crucian carps from the two testing water bodies during Spring–Summer period (May–June) that they grew asynchronously because of fractional spawning. It turned out that Goldfish, *C. auratus,* and Prussian carp, *C. gibelio,* had two fish eggs' generations. The acquired data and the low linear growth let us claim that the examined species belong to stunted forms (Movchan, Smirnov, 1983). Sexual maturation of the both species takes place in the second year of life. It turned out that the part of impuberal two years old individuals of all the examined Goldfish, *C. auratus*, was 25.9 % and the part of Prussian carps, *C. gibelio*, was 16.3 %.

The great number of impuberal individuals in the population of Goldfish, *C. auratus,* is caused by the fact that this species has a classical full fledged fractional spawning. The progeny is represented by all generations of hatchlings starting from Spring generation to Summer one. A part of juvenile Goldfish, *C. auratus,* are the late generation individuals. The observation of this species' progeny confirms it. The tiny fishes appeared in the test water body till the end of June (table. 1).

C. gibelio also plant eggs of all generations. During the pre-spawning period the bigger part of the female gonad was in III–IV maturation stage. During the spawning season, despite the fact that this species has no males, the prevailing number of unisexual crucian carps shed only the first portion of eggs that makes 20–70 %. The remaining fish eggs of stage III pass to stage IV, and then till Autumn slowly resorb, i. e. the female gonad after laying the first part of eggs of stage, III–IV, skips stage V, and gradually turns into stage VI–II. The confirmation of that is the presence in this species' progeny of first generation hatchlings only (table 1).

The reproductive female crucian carps that did not participate in spawning were discovered in the researched populations, i.e. they did not shed the eggs. The hard roe after stage IV, skipped stage V, and passed to stage VI–II. Among the two years old *C. auratus* the quantity of such individuals made 7.4 %, among *C. gibelio* it was 18.8 % (table 2). The percent of crucian carps of older age groups which did not participate in

Table 1. Hatchlings' body length in the researched water reservoirs (min–max values)

Month	Body length min–max, mm			
	N	*C. gibelio*	N	*C. auratus*
May	17	6.5–13	7	5–10
June	13	14–25	11	5–18.5
July	21	19–36	10	9–29.5
August	51	30–50	18	17.5–45.5

Table 2. Quantitative relation of not spent females of *C. auratus* and C. *gibelio*

Species	Age					
	2		3		4	
	N	n, %	N	n ,%	N	n, %
C. auratus	27	2 (7.4)	9	3 (33.3)	–	–
C. gibelio	16	3 (18.8)	21	8 (38.1)	13	4 (30.8)

Note. N — quantity of individuals in an age group; n — quantity of not spent females.

spawning is increasing. Among Prussian carp, *C. gibelio*, the quantity of such females is significantly larger than it is of the Goldfish, *C. auratus*. It ought to be remarked that the same peculiarity was discovered in female Amur carps during the studying of oogenesis and spawning processes (Gromov, 1987).

The hard roe analysis of the both species' females proved that the fish eggs' reabsorption of the young fishes is much quicker than of the representatives of older age groups. The hard roes of the researched two years old individuals passed all stages starting from maturity stage III–IV and ending with stage II within the four months period (April–July). Absolutely all the hard roes of the two years old Goldfish and Prussian carp individuals were on the stage II in June, i. e. after spawning the insignificant percent of remaining fish eggs completely diffused and the hard roe passed to the stage II. A little later this process finished among the three years old individuals. In late July only 44.4 % of all researched *C. auratus* spawned completely. Among the polyploid *C. gibelio* females the share of such individuals was 19.1 %, and among the four years old ones it was only 7.69 %. The older a zooid is the more time it needs for the completion of remaining fish eggs' reabsorption. Besides the full reproduction process takes Goldfish less time than it takes Prussian carp. It may be related to spawning process peculiarities of *C. gibelio*. This species has no distinct spawning process because it has no its own males and because of the restricted number of spermium-donor-species' individuals — crucian carp, *C. carasius*.

The three years old individuals of *C. gibelio* finish the reabsorption of remaining fish eggs completely in early August, the four year old individuals — in late August. This process is complicated and long for the individuals which did not participate in reproduction process and did not shed the eggs. The intermediary stage VI–II for such crucian carps takes place in early September.

The individual fecundity of female crucian carps increases proportionally to their length, body weight and age (table 3; fig. 1). The comparative analysis of the two species of crucian carps belonging to different age groups also discovered some differences. It turned out that individual fecundity of Goldfish, *C. auratus,* females is significantly higher than it is of Prussian carp, *C. gibelio*. The credible differences were found between the three years old ($p = 0.0004$), four years old ($p = 0.015$) and five years old ($p = 0.004$) age groups of the two species. The level of differences among two years old individuals of the two species turned out to be uncertain ($p = 0.1$). Apparently it is related to the fact that the populations of Goldfish, *C. auratus*, at the age of two years consist of individuals belonging to different generations. The latest generation of crucian carps is represented by small-scale individuals with rather low individual fecundity; it had a corresponding impact on the results.

Table 3. Dependence of individual fecundity of two species of *Carassius* on age, size and weight

Species	Age	N	Body length, mm				Body weight, g				Mass of egg, g				Individual fecundity, n			
			min	max	M	± m	min	max	M	± m	min	max	M	±m	min	max	M	± m
C. gibelio	1	7	51	82	61.1	1.2	1.44	7.52	3.4	0.6	–	–	–	–	–	–	–	–
	2	81	75	119	95.3	0.3	5.22	18.77	11	0.2	0.46	1.8	1.05	0.1	880	6,120	2,630	4.1
	3	76	84	137	115.17	0.3	8.27	36.29	20.01	0.3	0.97	3.12	1.66	0.1	990	10,608	3,234	5.0
	4	44	108	158	134	0.5	14.77	58.47	33.66	0.5	1.01	9.11	4.23	0.2	1,434	20,498	8,241	8.7
	5	16	132	161	150	0.8	28.58	57.87	45.89	0.6	3.05	9.45	5.82	0.4	4,118	16,765	10,107	16.3
	6	2	158	165	161.5	–	52.94	63.46	58.2	–	7.59	9.1	8.35	–	15,451	18,525	16,988	–
C. auratus	1	6	42	83	74.1	1.4	0.79	7.73	5.41	0.6	–	–	–	–	–	–	–	–
	2	110	72	118	87.84	0.3	4.3	22.49	9.12	0.2	0.17	2.24	0.92	0.1	1,586	6,345	3,446	3.5
	3	15	91	144	120	0.9	9.45	43.5	24.79	0.7	1.36	4.87	3.12	0.3	5,829	20,971	13,280	21.0
	4	5	116	146	136	1.5	19	41.84	35.71	1.4	1.6	8.23	5.42	0.8	2,055	35,241	22,545	51.2
	5	1	–	–	149	–	–	–	50.8	–	–	–	7.31	–	–	–	25,118	–
	6	1	–	–	193	–	–	–	110.65	–	–	–	23.54	–	–	–	80,821	–

It is worth noting that the figures of linear growth, body weight and individual fecundity of the researched crucian carps were different from average data related to the same species in Ukrainian water bodies (table 3). At an average in Ukraine the two years old individuals with body length 18.7 cm have the individual fecundity level at 57,2 thousand fish eggs, three years old ones with body length 24 cm — 131,5 thousand. (Movchan, Smirnov, 1983). This data confirms the assumption that the crucian carps from the test water bodies that have low linear growth index can belong to stunted forms.

In some degree with the increase of individual fecundity of a Goldfish, *C. auratus*, the relative fecundity increases too (table 4). The situation is opposite regarding Prussian carps, *C. gibelio* — with the increase of years the relative fecundity figures decrease. The differences in relative fecundity figures of the two species among the two years old individuals ($p = 0.0023$), among the three years old individuals ($p = 0.0022$), and four years old ones ($p = 0.005$) turned out to have high confidence level.

The fish eggs' quantity on stage III–IV in 1 gram was uneven in the same size ovaries of the two species (table 5). The Goldfish, *C. auratus*, has more fish eggs in all age groups than the Prussian carp *C. gibelio*. The difference in fish eggs number in a quantity weighed for analysis of the researched crucian carps among the two years old individuals ($p = 0.0044$), the three years old individuals ($p = 0.01$) and four years old individuals ($p = 0.0006$) turned out to have high confidence level. It ought to be remarked that smaller number of fish eggs in the Prussian carp's ovaries is related to their bigger size, since this species is a triploid one. That is why there are three sets of chromosomes in an egg cell instead of one. This consistent pattern can be observed according to cytometry data. The red blood cells' surface

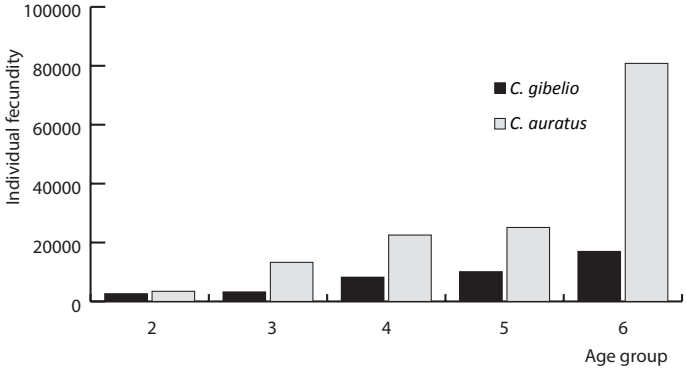

Fig. 1. Individual fecundity of *C. auratus* and *C. gibelio* females in age groups.

Table 4. Relative fecundity comparative analysis of *C. auratus* and *C. gibelio* females

Species	Age	N	Relative fecundity,			
			min	max	M	± m
C. gibelio	2	81	117	440	261	1,0
	3	76	99	390	243	0.9
	4	44	97	384	232	1.4
	5	16	106	290	213	2.0
	6	2	–	–	292	–
C. auratus	2	110	114	578	361	1,1
	3	15	208	825	503	4.2
	4	5	560	713	636	4.5
	5	1	–	–	432	–
	6	1	–	–	734	–

Table 5. Correlation of hard roe of two species' females in different age groups

Age	Quantity of eggs in the test portion 1 g				
	N	*C. gibelio*	N	*C. auratus*	p
2	22	2,669 ± 5.3	19	3,370 ± 1.5	0.0044
3	17	2,394 ± 4.4	8	3,620 ± 11.4	0.01
4	13	2,116 ± 7.0	5	3,333 ± 3.4	0.0006
5	8	2,100 ± 10.2	1	3000	

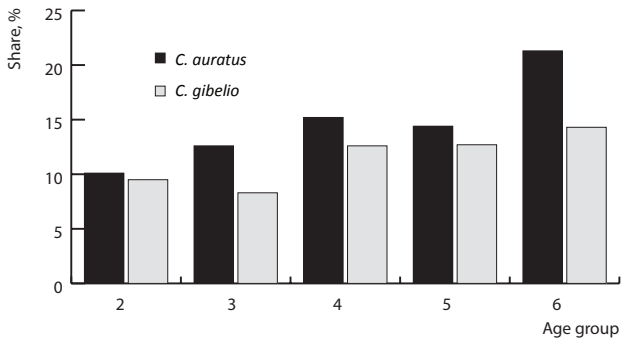

Fig. 2. Percentage ratio of hard roe and body weight of *C. auratus* and *C. gibelio* females.

area of triploid Prussian carps is 1/3 larger than it is of diploid Goldfish (Goriunova, 1960; Cherfas, 1966; Sezaki, Kobayashi, 1978; Shimuzu, 2003).

The reduction of fish eggs quantity in 1 gram from the youngest to older age groups of the two researched species is related to the increase of females' body size which results in proportional egg cells' increase (table 5).

After analysis of both species' reproductive products it turned out that proportion of fish eggs' weight to females' body weight had a great difference. In the age groups comparison the diploid *C. auratus* females have larger weight of ovaries than triploid *C. gibelio* females. I. e. despite the fact that *C. gibelio* individuals have less fish eggs than *C. auratus* in equal quantity weighed for analysis, it turned out that the weight of reproductive products of the both species' females of the same age is different. *C. auratus* females turned out to be more fertile (fig. 2).

Conclusion

As a result of conducted research it was discovered that Prussian carp, *C. gibelio*, individuals had smaller breeding potential and in case of coexistence with Goldfish, *C. auratus*, had to recede under the influence of the other species. The reason for that was low individual and relative fecundity level, great number of reproductive individuals which did not

participate in reproduction process, smaller number of fish eggs in equal size ovaries with Goldfish, *C. auratus*, low percent of laid fish eggs and correspondingly initiated fish eggs by sperm-donor-species Goldfish, *C. auratus*, or crucian carp, *C. carasius*. All the mentioned above processes led to small in number progeny of the gynogenetic species *C. gibelio*.

Despite the newly acquired data which proves the losing position of the unisexual species when it coexists with the digeneous one, there is a set of open questions. It is not known up to now under what circumstances after the settlement on digeneous species *C. auratus* in natural water bodies, the Prussian carp individuals completely disappear from the water body within a few years. According to our data the transformation of purely unisexual population of Prussian carp into digeneous one takes only three or four years (Mezhzherin et al., 2007; 2015). Considering the fact that unisexual females borrow part of other species' males for their own reproduction, the transformation of crucian carps' settlement structure should have taken much time and come in the end to balanced state regarding the quantitative ratio.

A great interest is stirred by the circumstances and reasons, that allow a little group of digeneous crucians, which settled into a water body inhabited by a pure triploid species population, to cause the irruption just in a few populations and relatively soon to dislodge the unisexual species. It is quite possible that interacting of triploid females with Goldfish males during the spawning has some complexities and problems, which influence the percent of triploid species' impregnated roe. Apparently Goldfish males prefer the females of their own species during spawning, and the combined spawning with triploid Prussian carp females happens on an occasional basis.

It should be added that complicated reproduction ways like pseudogamy or parthenogenesis can not provide effective reproduction, which can result in numerous progeny. Perhaps for that reason *C. gibelio* took fancy to small isolated water bodies, where it makes small populations, backed up by successful spawning with a single male of the crucian carp, *C. carasius*.

As a result there is a tendency in Ukrainian water bodies to formation of homogeneous settlements consisting exclusively of adventitious species — digeneous Goldfish, *C. auratus*. The appearance of *C. auratus* in an isolated water body will inevitably result in extinction of pre-existing population of *C. gibelio*.

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Received 27April 2016
Accepted 5 December 2016