

UDC 575.2:597:551 STRUCTURE AND DYNAMICS OF CRUCIANS' SETTLEMENTS (CYPRINIFORMES, CARASSIUS) IN WATER SYSTEMS OF EASTERN UKRAINE

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> Structure and Dynamics of Crucians' Settlements (Cypriniformes, Carassius) in Water Systems of Eastern Ukraine. Mezhzherin, S. V., Kulish, A. V., Kokodiy, S. V. — The analysis of present-day crucians' settlements in water systems of Eastern Ukraine designated the predominance of the digeneous Goldfish, C. auratus, in the region, the number of which made 78.7 %, from the total number of the examined representatives of the genus. The second group consists of gynogenetic Prussian carps, C. gibelio (14.3 %); it is represented by the clone biotype and recombinant individuals. Crucian carp, C. carassius (3.6 %), turned out to be rare and its number did not exceed the number of the caught hybrids C. auratus × C. carassius (3.4 %). The retrospective analysis of literature data and museum collections gave an opportunity to describe the changes in species composition of the genus which took place during the last 150 years. Within this period the crucian carp, which used to be the single and most common representative of genus Carassius (Jarocki, 1822) in the region, became nearly an extinct species. In the meanwhile the representatives of the group of species of Prussian carps, C. auratus + C. gibelio, which appeared in the region in the late 1960s, rapidly increased their number and became the most numerous fish of the Eastern Ukraine. The discovered tendency is not unique for the researched region; in general it reflects the European tendency for the crucian species. The reasons for that are rivers' regulation and destruction of bottomland ecosystems. The secondary factors for the elimination of C. carassius are the competitive relations of individuals representing both species and easy hybridization, during which the more numerous species C. auratus absorbs the rare C. carassius.

Key words: crucians Carassius, biological invasion, hybridization.

Introduction

The issue of invasive species and their interaction with elements of aboriginal fauna is a topical question of contemporary evolution zoology and ecology. A special interest is aroused by hybridization of invasion and aboriginal species with the following analysis of evolution and ecological consequences (Simberloff, 1996; Fitz-patrick, Shaffer, 2007; Vellend et al., 2007; Metcalf et al., 2008; Muhlfeld et al., 2014). This research is especially topical for the fish with common and diverse cross-species hybridization (Hubbs, 1955; Verspoor, Hammart, 1991; Dowling, Demarais, 1993; Vrijenhoek, 1994; Scribner et al., 2000).

One of the groups with extensive cross-species hybridization comprises the representatives of genus Carassius, the frequent interspecies crossing between them became the reason for the formation of numerous triploid and more rarely tetraploid forms which propagate themselves by means of gynogenesis (Cherfas, 1966; Golovinskaya at al., 1965; Kobayashi et al., 1977; Lin et al., 1980; Shimuzu et al., 1993; Murakami et al., 2001). The polyploid crucians of Eastern Asian origin are widely spread not only in East Asia but in Europe as well (Murakami et al., 2001; Takada et al., 2010; Mezhzherin, Kokodiy, 2010). Besides, in Europe the hybridization of aboriginal crucian carp C. carassius with invasive species and diploid Goldfish, C. auratus, and in exceptional circumstances even with triploid Prussian carp, C. gibelio, takes place (Hänfling et al., 2005; Mezhzherin, Lisetsky 2004; Papoušek et al., 2008; Mezhzherin at al., 2012). Yet the majority of hybrids which emerged with the participation of crucian carp are diploid, however the triploids are rather common too; the tetraploids are quite rare (Mezhzherin, Kokodiy, 2007; Mezhzherin at al., 2012). The hybridization intensity of C. auratus and C. carassius in some cases is so high that in particular water bodies the hybrids of C. auratus \times C. carassius prevail over parental species (Hänfling et al., 2005; Mezhzherin, Lisetsky, 2004; Mezhzherin at al., 2015). In particular in the Middle Dnipro basin the hybrids are caught by no means more seldom than C. carassius (Kokodiy, 2010). That is why it cannot be excluded that hybridization which is considered to be one of the species' extinction factor (Rhymer, Simberloff, 1996), along with water ecosystems' transformation, caused by rivers regulation and marsh drainage, became the reason for the range reduction and decrease in population of C. carassius in Europe (Freyhof, Kottelat, 2008; Sayer et al., 2011).

The Goldfish, *C. auratus*, was massively brought into the river systems of the Azov and Black Sea region of USSR in 1960s from the Far East during acclimatization of Far Eastern herbivorous fish. It was then that the irruption of this species took place in the lower reaches of the Dnipro where it was never encountered earlier (Ambroz, 1956), and later in large water storage basins of the Dnipro River (Demchenko, 1981). In the second half of 1970s it became the dominant species of the Danube's low course (Mezhzherin, 2008), and then it started to spread rapidly upstream (Holčík, Žitňan, 1978; Holcík, 2003; Lusk et al., 2010), eventually it invaded almost all the water bodies of Europe.

By the beginning of 1980s *C. auratus* — became one of the most numerous representatives of Ukrainian freshwater ichthyofauna. Furthermore, at that time already, there was not a single low land water system left, which was not settled by the Goldfish (Movchan, Smirnov, 1983). Its inhabitation places are not only the rivers, lakes and ponds but also small ephemeral impounded bodies, water filled open pit mines and coastal salt lakes. The populations of *C. auratus* appear even in salty water. In the Sea of Azov this species is common near the coasts in the desalted areas (Abramenko at al., 1997), where it is often caught along with the seafish. Expansion of *C. auratus* in Ukraine was simultaneous with the rapid reduction of *C. carassius*, which used to be the common commercial species of this river system before the regulating of the Dnipro with a range of dams (Mezhzherin, 2008). However, by the early 1960s it turned into a not numerous species and was not any more the part of fishing industry; now it is so rare that it was introduced into the Red Book of endangered species of Ukraine (Shcherbukha, 2009).

For the correct understanding of the situation and clearing up the reasons for changes of species composition of genus *Carassius* in European water bodies it is necessary to determine the contemporary settlement structure of crucian carps and establish the hybridization frequency of *C. auratus* and *C. carassius*, and to analyze the dynamics of crucians' species composition over extended period of time. The reasonably large river system is suitable for such kind of research, the system which ichthyofauna's data has been collected for a long period of time. The Siverskyi Donets should be considered such model river, which is the main water system of Eastern Ukraine. The upper reaches of some of the left tributaries of the Dnipro and small rivers that flow into the Sea of Azov are also located in this region. Within the last 50–60 years the considerable hydrologic changes have taken pace in all these river systems, those changes are related to regulation and creation of numerous artificially impounded water bodies. The first records related to fish fauna of the water basins of Eastern Ukraine are dated by the middle of the 19th century (Chernay, 1852). In the 20th century the researches were regular and were conducted since 1920s till 1990s (Solodovnikov, 1930; Fadeev, 1930; Maslovsky, 1940; Sakhno, 1940; Korotkiy, Kharitonova, 1958; Movchan, Smirnov, 1983; Denshchik, 1994; Movchan, 2005; Shandikov, Goncharov, 2008). This data and the obtained results during the first decade of the 21st century allow not only to determine the present-day structure of crucian community in the region but to implement the species dynamics analysis of this genus within the period of 150 years.

Material and methods

Material. The basis for studying the present-day settlement structure and hybridization were 30 selections of crucians from the water bodies and rivers within the territories of Krarkiv, Donetsk and Lugansk regions of Ukraine (fig. 1). The water bodies from which the selections were collected belong to catchment areas of the Siverskyi Donets (the Don), the Dnipro (upper course of the Samara River with confluents and the Vorskla River) and the rivers of the Northern Pryazovia. The selections were collected in 2006–2009. Overall 1135 individuals were analyzed, basically those were mature fish with the identified species exclusiveness, ploidy and sex, the number of fish scales in lateral line and gill rakers on the first gill arch were calculated. 675 of those were analyzed by electrophoretic and multilocus analysis. The material was collected during the whole season except winter time by the fishing gear permitted by applicable laws of Ukraine.

Taxonomia and species diagnostics. Crucian taxons' names herein are indicated according to (Kottelat, Freyhof, 2007). In this respect, the following diagnostics properties of mentioned authors were taken into consideration. Crucian carp, *C. carassius* (Linnaeus, 1758), digeneous species, the number of gill rakers 23–33, the number of fish scales in the lateral line 31–36. Goldfish, *C. auratus* (Linnaeus, 1758) — digeneous form, the number of gill rakers 38–47, the number of fish scales in the lateral line 26–31. Prussian carp, *C. gibelio* (Bloch, 1782) — unisexual triploid form, the number of gill rakers 37–52, the number of fish scales in the lateral line 29–33. Taking into account the fact that individuals belonging to species of *C. auratus* and *C. gibelio* are genetically and morphologically very close and were not distinguished earlier, during the literature analysis the individuals belonging to these species were named *C.* (superspecies *auratus*).



Fig. 1. The places of collecting crucian carp selections from the water systems of Eastern Ukraine.

Species and hybrid forms' diagnostics herein was implemented according to allozyme data and by ploidy determination via cytometry of erythrocytes on thin blood films. The reliability of these methods for distiguishing the species and hybrid forms was tested earlier (Mezhzherin, Kokodiy, 2010; Mezhzherin at al., 2012, 2015).

Museum collections. The series of crucians from the water bodies of Eastern Ukraine were studied in ichthyological collections of National Science and Nature Study Museum of National Academy of Sciences and Nature Museum of Kharkiv State University. In general 397 specimens collected from various places during the period since 1916 till 2008 were found (table. 1).

Biological analysis. The sex of crucians was determined visually by the male or female gonads.

Morphological analysis. During morphological description of species and biotypes of crucians the most relevant features for the diagnostics of genus *Carassius* were utilized (Mezhzherin, Kokodiy, 2009): the number of fish scales in the lateral line (*l.l.*) and the number of gill rakers on the first gill arch (sp. br.).

Cytometric analysis. It was conducted according to range of methodologies elaborated for crucians (Cherfas, 1966; Sezaki et al., 1997). The erythrocytes were measured with ocular (\times 16) with micrometer scale and objective (\times 100). On each smear preparation al least 30 normocytes were measured. The area size of blood cells was calculated in cubic micrometers like rectangle's area with cell's projection in it.

Allozyme analysis. The sample collection for electrophoretic analysis was conducted in laboratory environment, where the research fish were delivered; in most cases the fish were alive. The analyzed material consisted of muscle and blood samples of crucians. The preliminary researched tissue samples were kept in the test tubes with 10 % sucrose solution for 12 hours, afterwards they were centrifuged.

The electrophoretic analysis was conducted in 7.5 % polyacrylamide gel in tris-EDTA·Na₂-borate pH 8.5 system (Peacock et al., 1965). The structural proteins and enzymes that have diagnostic significance for crucians were researched, their qualitative variability was described in detail earlier (Mezhzherin, Lisetsky, 2005; Mezhzherin, Kokodiy, 2010; Mezhzherin at al., 2012): aspartate aminotransferase (*Aat-1, Aat-2*), glucose phosphate isomerase (*Gpi-1, Gpi-2*), lactate dehydrogenase (*Ldh*-B), non-specific muscle esterases (*Es-3*), structural muscle proteins (*Pt-1A, Pt-2*), hemoglobins (*Hb-A*) and transferrins (*Tf*). The gels' coloration for enzymes and crude protein was conducted routinely (Harris, Hopkinson, 1976).

Statistical processing. For database creation, statistical processing, visualization and graphic material creation the following software was utilized: Microsoft Office Excel 2003, SPSS 16.0, MapInfo 7.8 and Origin 6.1.

Results

Present-day structure of crucians' settlements. On the basis of morphological characters, cytometry and biochemical gene markers the Goldfish, *C. auratus*, Prussian carp, *C. gibelio*, Crucian carp, *C. carassius* and hybrids *C. auratus* \times *C. carassius* were identified.

The diploid Goldfish, *C. auratus*, was discovered in 28 selections and numbered 892 specimens (table 2); it made 78.7 % of the researched material. In the populations of this species the number of females prevailed, their share made 57.6 %. Among 3.8 % the

Piyor oyatama	Collection period,	Sample sizes		
River systems	years	C. auratus	C. carassius	
Siverskyi Donets River	1916-1941	-	16	
	1972-1973	20	222	
	1977-1981	6	15	
	1991	21	_	
Rivers of the Sea of Azov basin	1972-1973	5	33	
	1987-2003	20	_	
Samara River (Dnipro basin)	1930	-	9	
	2008	1	_	
Vorskla River (Dnipro basin)	1975-1980	20	9	

Table	1. Crucians'	samples from	the Eastern	Ukraine river s	vstems in zoolog	ical museums
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development of reproductive organs was not discovered, for that reason their gender was not identified.

The number of individuals of gynogenetic unisexual species *C. gibelio* was 162 specimens or 14.3 % from the total number of the researched fish. At the same time only one individual turned out to be immature, the rest of fish were identified as females. Due to the size of erythrocytes the majority of individuals are triploids (erythrocytes' area from 152 to 220 cubic micrometers); only one individual was identified as tetraploid (241 cubic micrometers). This species of crucians was discovered in 17 water bodies. Apparently it propagates itself by the range of pseudogamous reciprocities with males of *C. auratus* and *C. carassius*. Three biotypes according to genotypic combinations of allozyme loci were discovered within *C. gibelio* (table 3), it would be more correct to regard two of them as groups of biotypes.

The monotypic biotype "gibelio-1" was described earlier according to the set of specific allozyme markers (Mezhzherin, Kokodiy, 2010). The products of alleles of loci *Aat*-2¹¹⁰ are typical in electrophoretic spectra; they are missing in the gene pool of *C. auratus*. This biotype is represented in four water bodies and frequently by the numerous series. As a result its share made approximately 42 % of *C. gibelio* individuals which were analyzed at the allozyme level. All individuals had the same set of genotypes. It proves the clone nature and consequently good running of gynogenetic reproduction.

Sample (coordinates)	C auratus	C gibelia	C carassius	C auratus × C carassius
Busovava Bavine (50°20′ 37°15′)	2. ингиниз 2	11		
Hrvmuny Pavine $(50^{\circ}20^{\circ}, 57^{\circ}18^{\circ})$	2	21		
Liman Laka $(40^{\circ}33', 36^{\circ}31')$	35	21	_	_
$K_{\rm ruglova I alca} (49.33, 30.31)$	5	2	-	-
Homut Lake $(4924, 5035)$	5	5	0	3
$\begin{array}{c} \text{Homut Lake (49.5, 57.27.)} \\ \text{Descharges Device (40824', 27851')} \\ \end{array}$	22	_	_	2
Peschanka Ravine $(49, 34, 37, 51)$	23	-	—	—
Uskol Reservoir $(49°22, 37°33)$	19	12	_	_
Nitrius River $(49^{\circ}4, 37^{\circ}42)$	57	37	-	_
Poyma Lake (48°55°, 37°44°)	69	6	3	25
Lopuhovatoye Lake (48°53′, 37°51′)	11	-	2	1
Pozharnjoye Lake (48°53′, 37°51′)	-	-	15	-
Bakay Lake (48°53′, 37°52′)	38	2	-	-
Slepnoye Lake (48°52′, 37°37′)	46	-	-	-
Liman Lake-1 (48°50′, 37°34′)	20	-	13	4
Liman Lake-2 (48°50′, 37°34′)	16	-	_	_
Kazenniy Torets River (48°49′, 37°37′)	68	5	_	_
Krasnaya Dolina Ravine (48°52′, 37°32′)	52	19	1	1
Vinokurniy Pond (48°29′, 37°25)	17	2	1	_
Krivoy Toretz River (48°22′, 37°56′)	19	4	_	-
Kleban-Bykskoye Reservoir (48°26′, 37°42′)	96	2	_	_
Samara River (48°41′, 36°56′)	75	25	_	-
Mar'evsk Reservoir (48°33′, 37°10′)	31	7	_	_
Grishinka River (48°18′, 37°6′)	22	_	_	-
Volch'ya River (48°10′, 37°25′)	20	-	_	-
Karlov Reservoir (48°8′, 37°29′)	68	2	_	-
Chirchin Ravine (47°54′, 38°49′)	13	-	_	-
Kamyshevaha Ravine (47°47′, 38°34′)	14	1	_	_
Grusky Elanchik River (47°35′, 38°15′)	15	-	_	-
Pavlopol Reservoir (47°18′, 37°48′)	22	_	_	_
Kamyshevatka River (46°59′, 37°13′)	10	_	_	-
Total	892	163	41	39

Table 2. Crucians' settlements structure of river systems of the Eastern Ukraine

Individuals of biotype "gibelio-2" were encountered more often. 54 % of all C. gibelio individuals, analyzed at the allozyme level, belong to this biotype. It was discovered in 16 water bodies and was, as a rule, represented by small series of various genotypes (table 3). In its allozyme spectra there were found allele products peculiar to *C. auratus*. As a result individuals of biotype "gibelio-2" according to the markers' set "put on a mask" of C. auratus and can be identified to 100 % only by means of combination of allozyme analysis with cytometry. It is no coincidence that some researchers (Jakovlič, Gui, 2011; Qin et al., 2016), who used the DNA markers, which indicated the genetic identity of triploid and diploid crucians of complex C. (superspecies *auratus*) considered their appearance to be the result of autopolyploidy. Unlike "gibelio-1" biotype "gibelio-2" is polymorphic. It is notable that it is polymorphic at such an extent that in each water body zooids with their own genotype combination according to two most variable loci Aat-1 and Tf are encountered. Such extent of variability gives ground to assert that in the situation with "gibelio-2" the hypervariability takes place, it is common for many clone species (Mezhzherin at al., 2018). It is obvious that the reproduction of biotype "gibelio-2" is related to recombination. One can assume that in this case the insufficiently explored phenomenon of inheritance, which is common to triploid organisms, takes place; it combines clone reproduction and Mendel inheritance (Stöck et al., 2012). The phenomenon's mechanism works as follows: during semen-dependent unisexual reproduction separate loci, chromosomes or even whole genomes of an egg cell can be replaced by the corresponding structures of the genetic apparatus of a spermatozoon. The possibility of such phenomenon for crucians is confirmed by the fact that during gynogenetic breeding the introgressions of genetic material of spermatozoons take place (Yi et al., 2003; Tóth et al., 2005). The progeny of such breeding must have the recombinant genotype and it can be considered to be hybrid one. For the individuals of biotype "gibelio-3" the genotype with unique allele $Aat-2^{105}$ is common (table 3); it is not typical for the populations of *C. auratus*. In general four specimens with different genotypes of locus Aat-1 were discovered. All of them are from the same water body.

In most cases *C. gibelio* individuals were encountered along with *C. auratus*, what's interesting is that the number of *C. auratus* prevailed in such settlements with only one exception. The peculiarity of crucians of Eastern Ukraine is almost complete lack of unisexual settlements, consisting of *C. gibelio* individuals only; it is common for the Northern Ukraine and the Middle Dnipro (Mezhzherin et al., 2017). The only exception was the selection from the Kharkiv Region from the pond of the gulch Khripuny where the confluent of River Volchya runs through. It is worth noting the tendency of growing the share of triploid *C. gibelio* in the Northern boarders of the researched area (fig. 2).

In selections dated 2006–2009 the crusian carp, *C. carassius*, was rare. Only 41 specimens were found in 7 selections. As a rule it was a mixture to *C. auratus*. However in a small lake Pozharnoye in Donetsk Region located on the second river terrace the settlement of *C. carassius* was discovered. Individuals from this water body had small size and slender body form. Seven individuals of crucian carp had obsolescent generative organs. The female share made 47 %.

The number of hybrids of *C. auratus* × *C. carassius* turned out to be nearly the same as the number of crucian carp. 39 specimens were discovered in 6 selections. Judging by the size of erythrocytes, which had the same variation range as *C. carassius* the hybrids were diploids and belonged to both male and female sexes. 8 % did not have gonads with grown reproductive products; it corresponds to populations of *C. carassius*. The females made 58 %. According to heterozygous combinations of parental species diagnostic loci (*Ldh*-B, *Aat*-1, *Aat*-2, *Pt*-1B, *Es*-3) all of hem were hybrids F_1 .

The water bodies were diverse not only according to species composition of crucians, but according to the representation of peculiar species in them too. The researched selections can be divided into seven groups (table 4). Most commonly the water bodies are inhabited by *C. auratus* only or by *C. auratus* with small number of *C. gibelio*. The water

Biotypes	Aat-1	Aat-2	Tf	N
"gibelio-1"	100-100/110	100-100/110	aac	38
"gibelio-2"	100-100	100-100	ab	7
	100-100	100-100	ac	3
	100-100	100-100	aac	2
	100-100	100-100	adx	1
	100-100	100-100	a"c	1
	100-100	100-100	bc	1
	100-100	100-100	aa'	1
	100-100	100-100	bc'	1
	100/110	100-100	ab	3
	100/110	100-100	adx	4
	100/110	100-100	aa'c	4
	100/110	100-100	ac	1
	100/110	100-100	a'a''	1
	100/110	100-100	a'	1
	100/110	100-100	aac	1
"gibelio-3"	100-100/110	100/105	cbb'	2
	100-100	100/105	cbb'	2

Table 3. Genotypic structure and diversity of *C. gibelio* biotypes simultaneously investigated by three allozymic loci

Note. N — number pd specimens.

bodies inhabited by all four genetic types of crucians were much more rare, just like water bodies inhabited by Goldfish, crucian carp and hybrids. There were single cases of homogenous selections of crucian carp, Prussia carp or rapid dominance of the latter over Goldfish.

Morphological characters' variability. The comparison of various forms and biotypes of crucians which based on the number of gill rakers on the first arch proved that



Fig. 2. Changes in *C. gibelio's* share in the selections of crucian carps from Eastern Ukraine depending on geographical latitude.

opula- in type	Percentage of the species and hybrids					Numbers pd settlements $(n = 30)$	
P.d	C. auratus	C. carassius	C. gibelio	C. auratus × C. carassius	всего	%	
1	100				9	30	
2	$\geq 61 - 98$		$\leq 2-39$		11	36.7	
3	≥ 23-85	$\leq 1-27$	$\leq 6-26$	≤ 1−27	4	13.3	
4	≥ 54-82	$\leq 14-35$		$\leq 7-18$	3	10.1	
5	≤ 15		≥ 85		1	3.3	
6		100			1	3.3	
7			100		1	3.3	

Table 4. Distribution of crucians settlements according to population type

Note. The numbers indicate the limits of variation of the proportion of biotype individuals in joint settlements; the sign (\geq) indicates the biotype that dominates in the reservoir in number; the sign (\leq) indicates a biotype that is in the minority in number or in a subordinate position in the reservoir.

according to both characteristics they differentiate distinctly on the average values level and the variability limits in most cases transgress. (table 5). The exception is comparison of individuals of *C. auratus* with *C. carassius* basing on the number of gill rakers. The first species had minimum 40 gill rakers, the second species had maximum 35 ones; it provides reliable hiatus. The hybrids of *C. auratus* × *C. carassius* had middle value of this characteristics shifted towards *C. auratus*. At the same time the dispersion of values is from 30 till 40 gill rakers; for that reason it is impossible to utilize this attribute for unmistakable diagnostics of hybrids. As it was expected (Kottelat, Freyhof, 2007), *C. gibelio* is characterized by the increase of the middle value of gill rakers compared to *C. auratus*. However these attributes can not be utilized for differentiation of these species due to wide transgression. The average number of gill rakers of different biotypes of *C. gibelio* is not the same either (table 5). The individuals of biotype *C. gibelio*-1 have positively more of them than *C. gibelio*-2, it was mentioned earlier regarding populations from the Dnipro basin (Mezhzherin, Kokodiy, 2009).

The number of fish scales in the lateral line is even less reliable attribute for differentiation of species and biotypes of crucians than the number of gill rakers, however it is reliable on the middle level, especially when comparing *C. auratus* with *C. carassius* (table 6). At the same time the hybrids of *C. auratus* \times *C. carassius* according to this attribute hold the middle position and differentiate positively from the parental species.

Dynamics of species composition. The species composition history is based on the literature, museum collections and personally collected data; it covers the period of 150 years.

The first authentic information regarding species composition of crucians from the region can be found in the Eastern Ukraine's fauna reviews of the first half of the 20th century (Chernay, 1852). At that time this region was inhabited by numerous representatives

Species or hybrid		М	SD	Lim	n
C. auratus		47.13	3.53	37-56	892
C. gibelio (total)		49.46	3.71	40-56	163
	"gibelio-1"	52.05	2.26	48-56	48
	"gibelio-2"	47.91	3.51	40-54	103
	"gibelio-3"	53.50	0.58	53-54	4
C. carassius		28.26	3.57	22-35	41
C. auratus × C. carassius		39.58	4.08	30-48	39

Table 5. Comparison of various forms and biotypes of crucians based on the number of gill racers variation

Note. M — means, SD — sample deviation, Lim — limits of variation, N — sample sizes.

Species or hybrid		М	SD	Lim	n
C. auratus		31.16	0.92	28-33	892
C. gibelio (total)		31.24	0.81	29-33	163
	"gibelio-1"	31.46	0.65	30-33	48
	"gibelio-2"	31.39	0.89	29-33	103
	"gibelio-3"	31		31	4
C. carassius		34.46	0.71	33-36	41
C. auratus × C. carassius		32.03	0.63	31-33	39

 Table 6. Comparison of various forms and biotypes of crucians based on the number of fish scales in the lateral line variation

Designations are the same as in the table 5.

of the so called *Cyprinus carassius*. This species had the following features: circular body profile, dark green with golden shade body color, bright cherry-colored fins, 33 fish scales in the lateral line, tailfin with small emargination. All these features prove that they related to *C. carassius* in modern sense. There is no information in the publication regarding the presence of another species of crucian, *Cyprinus gibelio*, in the region, which was mentioned at that time (Kessler, 1887) for water systems of the right bank Dnipro.

The Siverskyi Donets. In 1920th the first fully-fledged ichthyological research was conducted in the river-bed of the Siverskyi Donets (Solodovnikov, 1929, 1930). Only the common crucian carp *C. carassius* was referred to as the most frequent species in the catches. The further research of crucians from the Siverskyi Donets' bottomland was conducted in 1935/36 years (Maslovsky, 1940). All the collected specimens after calculation of gill rakers, the number of which ranged from 23 to 35, were identified as *C. carassius*.

All species composition related data published in 1940s (Sakhno, 1940) and 1950s, was based on the calculation of the gill rakers. At the same time in the selections from river bed of the Siverskyi Donets only individuals of *C. carassius* were discovered. Moreover at that time in the fish ponds along with the carp (*Cyprinus carpio*) only *C. carassius* was bred.

In 1960s the regulation of the Siverskyi Donerts' River bed started. The ichthyofauna research conducted at the end of this decade in the river bed and confluents of the lower course indicated the presence of representatives of *C*. (superspecis *auratus*) which were represented at that time in equal proportion with *C. carassius* (Troitsky, 1974). In 1970–1973 the populations of *C*. (superspecies *auratus*) increased their number, and their share in the catches from the Siverskyi Donets made approximately 78 % (Movchan, Smirnov, 1983).

Since 1980s the representatives of *C*. (superspecies *auratus*) became the most numerous commercial species in the Siverskyi Donets. According to the information available in early 1990s, the proportion between the number of *C. carassius* and *C*. (superspecies *auratus*) in the catches from confluents and river bed of the middle course was 1:5.4 (Denshchik, 1994). At the same time in the overflow-land water bodies of the upper course of the Severskiy Donets there were settlements consisting of individuals representing only *C. carassius* (Nazarov, 1995).

On the basis of Seversky Donets' ichthyofauna reviews published in the early 2000s (Movchan, 2005; Shandikov, Goncharov, 2008), one can make a conclusion about continuing disappearing of *C. carassius in* in the main river bed at a time when the number of representatives of *C.* (superspecies *auratus*) grows. The populations of crucian carp are preserved in confluents and separate lakes and their number became insignificant.

During the research conduction in 2006–2009 the share of crucian carp reached its lowest number in history (fig. 3, a), it made nearly 5 %.

Rivers of Pryazovia. The available current data regarding the species composition of genus *Carassius* in the rivers of Pryazovia proves that the tendency is the same as the one in the Siverskyi Donets (fig. 3, b). The first individuals of *C*. (superspecies *auratus*) were caught in the rivers of this region in 1950s (Demchenko, 2009). Afterwards the Goldfish



1991

2006-09

Fig. 3. Share correlation dynamics of individual of crucian carp, *C. carassius* (light-grey), and representatives of complex *C.* (superspecies *auratus*) (white) within the structure of genus *Carassius* during the last 80–100 years: 1 -basin of the Siverskyi Donets River, 2 -rivers of the Northern Pryazovia; 3 -river heads of the left confluents of the Dnipro River.

1916-41 1972-73 1977-81



started to appear in all the river basins of the region, increasing its number and replacing *C. carassius*, which used to be the autochthonous species in those rivers (Belling, Himmelreich, 1940). At the present moment the crucians representing group *C.* (superspecies *auratus*) are common not only in all the fresh-water systems but in the coastal desalinated areas of the Sea of Azov (Abramenko at al., 1997).

The confluents of the right bank Dnipro. There is museum material related to crucian carps from the confluents of the rivers Psel (1930) and the Vorskla (1975). In total 18 individuals of *C. carassius*. The later collections from the upper Vorskla (1980) and the Samara River (2008) comprise 21 specimens representing *C.* (superspecies *auratus*). The tendency is the same as the one in the other rivers of the Eastern Ukraine (fig. 3, c).

The generalization of museum materials and personal data, acquired during the field research, gave an opportunity for quantitative evaluation of changes of species composition of crucians since 1914 till 2009. Initially all water systems of Eastern Ukraine were represented only by *C. carassius*, which was gradually replaced by the representatives of *C.* (superspecies *auratus*). At present time *C. carassius* is preserved only in the basin of the Siverskyi Donets River, its share made 5 % from the collected individuals, while in the rivers of Pryazovia and in the Samara River (left confluent of the Dnipro) this species could not be found. Utilization of all available data, including literature, gave an opportunity to make the mapping description for the dynamics of crucian areas in the region within the indicated period (fig. 4).

Discussion

Hydrological changes of the Siverskyi Donets River and depression of crucaian carp's populations. On the basis of the collected data one can assert that crucian carp is an endangered species in the region. Its populations are preserved occasion-

%

100

80

60

40

20

0





ally in very small isolated water bodies tending to complete drying out. At the same time the invasion species C. auratus, which appeared in the region in the late 1960s has become the most numerous commercial species of Eastern Ukraine. The question regarding the reasons for such fundamental change demands special discussion. The factors for extinction of one species and wellbeing of the other can be either the direct interaction of species (competition and hybridization) or the changes of hydro-ecology systems. Anyway, the main reason should be considered the changes of landscapes and ecosystems caused by the regulation of rivers, the Siverskyi Donets in particular. It follows from the fact that the depression of C. carassius in the Ukrainian water systems started before the C. (superspecies auratus) became a widely spread species and could become a competitor to C. carassius. In 1930s the crucian carp was a common commercial species in the Dnipro River. In 1946– 1960 about 20-70 tonnes of crucian carps were caught annually. After the creation of Kakhovka and Kremenchug reservoirs on the Dnipro the catches of this species in 1961–1965 were reduced to 8 tonnes annually, and in the second half of this decade after the irruption of Goldfish in the Dnipro, C. carassius stopped to be considered as a commercial species (Mezhzherin, 2008).

The similar situation has arisen in the Siverskyi Donets, where the reduction of crucian carp's number took place before the irruption of C. (superspecies *auratus*). The decrease of populations of *C. carassius* coincided with the mainstream's regulation of the Siverskyi Donets in 1958–1964, and the representatives of C. (superspecies *auratus*) appeared here in sufficient scale only in the late 1960s. It is well known that the construction of the two hydro-electric power stations on the Siverskyi Donets resulted in the reduction of the water content and the river flow almost by one third. Up to 1967 the annual river flow was 4.93 km³, perennial annualized river flow rate in the river bed was 156 m³/s, and the perennial modulus or flow was 2.13 l/s from km². Similar hydrologic figures, collected within the period 1985–2011, they are as follows. Annual river flow and perennial annualized river discharge in the river bed of the Siverskyi Donets reduced by 24 % and made 3.76 km³ and 119 m³/s correspondingly. At the same time the long-term annual average modulus or flow reduced by 34 %, and made 1.62 l/s km² at this period. It would seem that for such eurybiontic fish as crucians, which avoid fast river currents, their slowdown should have become the favorable factor, but this did not happen. The habitat changes turned out to be favorable for C. (superspecies *auratus*) and very inconvenient for C. carassius. Obviously the reason for that is the current velocity by itself. The reason for aboriginal species' extinction was destruction of fluvial plain, flooded out by the spring floods, which used to be its spawning bed. After regulation of the Siverskyi Donets the maximum river discharge during seasonal flood in 1984-2010 comparing to the period before regulation decreased more than 3 times from 1068 to328 m³/s, consequently the rising of water into flood land decreased. In 1960s the average duration of seasonal flood on the Siverskyi Donets used to be 72.5 days, and at the end of the 20th century its duration reduced to 16.5 days. It caused the rapid change of the flood land's landscape, drying out of flood land lakes which are the main reservation of crucian carp. And the representatives of C. (superspecises auratus) are indifferent to changes in flood land, and the reason for their well-being is just the reduction of current velocity and emerging of any creeks with low flowing and waterbodies with slack water.

General tendencies of crucians' settlements in Europe. Common crucian carp inhabits trans-palearctic species area, spreading through the Northern part of Eurasia from the British Isles in the West through Siberia to the rivers of the basin of the Sea of Okhotsk. The vast area of species and the lack of threat throughout the most of the area determine the leaching resistance of the species *C. carassius*. It is no coincidence that in the Red List of International Union for Conservation of Nature Resources the common crucian carp has the least concern status (Freyhof, Kottelat, 2008). Nevertheless, in the Western border of its area — across the most of European territory — it definitely became the endangered species and a range of researches prove that. During the research of 40 Brit-

ish lakes in the County of Norfolk, which were inhabited by numerous representatives of common crucian carp in 1970–1980s, it was revealed that this species was preserved only in the 11 ones (Sayer et al., 2011). In addition its number was significantly lower than the one of the immigrant — Goldfish, *C. auratus*. This situation is not unique for the British water bodies. It is no coincidence that Natural History Museum in 2010 published on its web site the information that this species belonged to the endangered species of Europe. In that respect the situation related to this species in Eastern Ukraine in not exceptional, it confirms the general tendency, which can be clearly seen in the neighboring river basins of the Sea of Azov and the Black Sea basin and in Europe in general.

It is clear that such broad-scale deformations of the crucian settlements were most probably caused by the changes of European hydro-ecological systems which resulted from rivers' regulation. It caused the changes and disturbance of bottomland biotypes' structure, and in particular drying out and degradation of bottomland lakes. The irruption of Goldfish, *C. auratus*, was a circumstance of no small importance, at present time this species is massively represented almost in all regions of Western and Central Europe, where the common crucian carp can still be encountered.

One can assume that one more reason for the gradual extinction of *C. carassius* could be the hybridization with the adventitious species *C. auratus* (Hänfling et al., 2005; Mezhzherin, Lisetsky, 2004; Papoušek et al., 2008). Moreover, the hybridization is so wide-scale that in certain cases, including the water systems of Eastern Ukraine, the frequency of occurrence of hybrids is higher than of *C. carassius*. Apparently some extra factors should be added to that: drying out of flood-plain lakes is particular years; water quality impairment; trophic and spawning competition with immigrant cyprinoid species; reclamating works and predators.

One can predict that, under the present circumstances of water ecosystems' transformation, crucian carp, *C. carassius*, will reduce its species area and number of populations in future, and the representatives of *C.* (superspecies *auratus*) will increase their number and spread across all Europe.

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