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REPRODUCTIVE POTENTIALS OF DIPLOID AND POLYPLOID REPRESENTATIVES OF THE GENUS *COBITIS* (CYPRINIFORMES, COBITIDAE)

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Reproductive Potentials of Diploid and Polyploidy Representatives of the Genus *Cobitis* (Cypriniformes, Cobitidae). Mezhzherin, S. V., Salyy, T. V., Tsyba, A. A. — Comparison of reproductive parameters in several *Cobitis* forms with different ploidy shows that the maximum fertility is found in diploid *Cobitis*, the triploids are less fertile and the tetraploids even less fecund. The latter reach maximum values of size and weight indicators but minimum number of eggs, the smallest size of the ovaries but the biggest eggs. Nevertheless, the combined reproductive potential of polyploid females is higher than of diploid spiny loaches with equal ratios of males to females in populations. The obvious reasons for the successful expansion of polyploid individuals of the spiny loaches are both their higher fertility at the population level and the changes in environmental conditions associated with the regulation of all major rivers in Ukraine in the 1960–1970s.
Key words: *Cobitis*, fertility, polyploidy, invasion.

Biological invasion — the spread of a "species" into an area that was not previously occupied by it — can have strong effect on ecosystem structure and function. A special case of invasion is the expansion of gynogenetic biotipes of spiny loaches of the genius *Cobitis* Linnaeus, 1758 from the Danube basin to river systems of Eastern Europe. It is known that most of European triploid and tetraploid spiny loaches of this genus (Bohlen, Rab, 2001; Bohlen et al., 2002; Janko et al., 2007; Mezhzherin, Pavlenko, 2010) occurred as a result of serial hybridization of Danube spiny loach *C. elongatoides* Băcescu, R. Mayer, 1969 that lives in Danube river system together with the spined loach *C. taenia* Linnaeus, 1758 and the *C. tanaitica* Băcescu, R. Mayer, 1969 (originating from Don River). The spined loach's range covers northern parts of European range of the genus *Cobitis*, while *C. tanaitica* occurs to the south, mostly downstream in rivers of the Azov-Black Sea basin, it is also found in all of the Danube (Bohlen, Rab, 2001; Bohlen et al., 2002; Janko et al., 2007). Thus the contact and hybridization between these species take place in Danube basin and bordering river systems of the North Sea rivers. *C. elongatoides* and *C. tanaitica* hybridize downstream (Mezhzherin, Pavlenko, 2007) and in middle reaches (Janko et al., 2007). In the case of hybrids of *C. elongatoides* × *C. tanaitica* backcrossing, triploid biotypes *C.* 2 *elongatoides — tanaitica* and *C. elongatoides* — 2 *tanaitica* occur, while triploid biotypes *C. elongatoides — tanaitica — taenia* and *C. elongatoides — tanaitica* — 2 *taenia* form upstream in the basin at the point of contact of *C. elongatoides* and *C. taenia* (Janko et al., 2007). Therefore, it is quite correct to consider the Danube to be the place of origin of most European triploid biotypes of spined loaches, from where they have begun to invade other European river systems. Now unisexual polyploid spined loaches are common in Dniester basin, Southern Buh basin, Dnipro basin (Mezhzherin, Lisetskaya, 2004; Mehzhzherin, Pavlenko, 2010) and Volga basin (Vasilyev, 1985). There they reproduce by way of gynogenesis with males of local diploid species. Unusually, polyploid forms are now dominant (more numerous) than diploids both in the Danube proper and in mixed populations in middle reaches of Dnipro, in Dniester downstream, in Seversky Donets River. As a rule they constitute 90–95 % of population. As such, polyploids ousted local diploid spe-

cies to the periphery of the living space. Thus, homogenous populations of C. *elongatoides* are represented in the foothill parts of the Carpathians rivers), homogenous populations of *C. taenia* are preserved in Ukraine in small rivers and isolated lakes, and populations of *C. tanaitica* occur in small endangered rivers of the northern coast of the Azov Sea (Mezhzherin, Pavlenko, 2009).

The polyploids' expansion is confirmed by analysis of ichthyologic collections of D. E. Beling (collected from Middle Dnieper in 1920–1930-s, currently in collections of NNPV NAS of Ukraine). It was established (Mezhzherin, Chudakorova, 2001) that populations of spined loaches in Middle Dnipro then consisted of males and females in roughly equal proportions. But since 1960s, males have been almost totally absent in spined loach populations and now their fraction is 1–2 %. This means that in the second half of XX century, after the damming of the Dnieper stream, triploid female biotypes began to dominate in the spined loach populations. It is still unknown whether this is a recent invasion or an explosive increase of numbers in new life conditions.

In any case, the question arises of the reasons for the numerical dominance of polyploids. Since diploids and polyploids occur simultaneously without exhibiting explicit biotopic preferences, the ecologic factors are hardly to blame. Thus the reasons for the rapidly increasing numbers of triploid biotypes and oppression of local diploid species can lie in their unequal reproductive potentials and competitive advantages. Particularly, triploids may have varying individual fecundity compared with diploids. To verify the possibility, this study was conducted.

Material and methods

Materials for the study were serial samples of spined loaches collected in May–June of 2015, and June–September of 2016 in two small rivers of Middle Dnipro basin: Stugna River (right tributary of Dnipro) near Motovylivka Village (50°10′06.88″ N 30°06′03″E) and Oster River (tributary of Desna River) directly within Nezhin City (51°31′10.3″ N 31°51′55.4″ E). Altogether 83 females from the Stugna River and 199 from Oster River were analyzed.

Biological analysis of spined loaches is conducted according to conforming recommendations (Pravdin, 1966). The fishes were measured by total body length (L), standard length (l), body weight (W), yastik weight (Wo). Small samples of eggs were collected from each specimen, to count eggs. Then average egg mass per sample was calculated as sample weight to number of eggs ratio, thus allowing estimating the number of eggs per yastik. Also, egg amounts were assessed visually along with the presence and quantity of parenchyma in ovaries.

Biotype identification of spined loaches was done by two techniques: allozyme analysis of loci *Aat*-1 and *Mdh*-1A, and cytometry (measuring erythrocyte nucleus area). Said techniques of electrophoresis and cytometry are described in detail in previous works (Mezhzherin, Chudakorova, 2002).

Statistical processing was carried out using the program Statistica.7.

Results

Population genetic structure. Spined loaches populations in two rivers consisted of diploid and polyploids, and biotype ratios for the rivers are notably different (table 1). Population of Stugna river is mostly comprised of diploid *C. taenia* (72 % of studied specimens). Polyploid biotypes are represented by two groups: the first constitutes 5 % of them and is comprised of *C.* 2 *elongatoides — tanaitica* triploids, and the second group consists of (*C. elongatoides* — 2 *taenia*, *C. elongatoides — taenia — tanaitica*) triploids and (*C. elongatoides* — 3 *taenia*, *C. elongatoides — 2 taenia — tanaitica*) tetraploids, the latter comprising 23 %. In the population of Oster river, diploid *C. taenia* specimens are very rare, only 4 % of all. In this river system, such triploid and tetraploid biotypes were dominant: *C. elongatoides* — 2 *taenia*, *C. elongatoides — taenia — tanaitica*, *C. elongatoides* — 3 *taenia*, *C. elongatoides — 2 taenia — tanaitica*.

Seasonal dynamics of reproductive characteristics. It is known (Movchan, 1988), that spined loaches are fishes with lengthy batch spawning, the activity peaking at the end of May — in the beginning of June. Indeed, the largest yastiks filled to the maximum by eggs are observed in this time. Specimens with small and large eggs are frequent then. Mid-summer, the percentage of egg-bearing females drops so that in the beginning of August they are not found at all. New eggs in spined loaches begin to develop in September.

Table 1. Number of specimens of various biotypes of *Cobitis* spined loaches

River	Year	Biotypes		
		C. taenia 2n	*C.* 2 *elongatoides — tanaitica* 3n	*C. elongatoides* — 2 (3) *taenia*, *C. elongatoides* — 1 (2) *taenia* — 1 (2) *tanaitica* 3n, 4n
Stugna	2015	36	6	14
	2016	32		5
Oster	2015	7		150
	2016	3		81

Note. n — number of chromosome sets.

Specifically, dynamics of analyzed reproductive characteristics in May–July is shown in tables 2–3. Total number of eggs in yastiks during this period reduces more than twice. For example, in Stugna River population, average number of eggs per yastik in the first decade of June is 1137, and in the third decade it becomes 585. During this period, percentage of females with eggs decreases to 68.4 %. In Oster River population the changes of reproductive characteristics are more dynamic. In May, 2015 average number of eggs per female is maximum (672 eggs) and in June it falls to 143 eggs per female, and the percentage of females with eggs is but 26 %.

Accordingly, the decreasing numbers of eggs are followed by decreasing ovaries' weight. And since the end of June, eggs are absent in some ovary samples, being gradually substituted by parenchyma. As a result, to the end of spawning season, significant portions of yastiks are comprised by parenchyma, leading to falsely large egg sizes.

Table 2. Seasonal changes of reproductive characteristics in female spined loaches in the Stugna River

Year	2015						2016					
Day	May 27			June 16			June 9			July 22		
	n	M	SE	n	M	SE	n	M	SE	n	M	SE
N	27	880.1	102	18	674.5	68.6	18	1137.7	108	11	585.7	115.5
Wo, g	27	0.55	0.05	18	0.42	0.04	18	0.56	0.04	11	0.22	0.04
w, mg	27	0.84	0.13	18	0.73	0.07	18	0.55	0.05	11	0.78	0.29
%	28	96.4		20	90		19	95		16	68.7	

Note. N — calculated number of eggs per yastik; Wo, g — weight of yastik, w; mg — calculated egg weight; % — portion of egg-bearing females; n — number of studied females; M — average value; SE — standard error.

Table 3. Seasonal changes of reproductive characteristics in female spined loaches of Oster River

Year	2015											
Day	May 18			June 9			June 23			July 18		
	n	M	SE	n	M	SE	n	M	SE	N	M	SE
N	31	672. 9	98.6	18	332.9	48	15	181.9	33.8	16	143.1	20.5
Wo, g	32	0.53	0.06	18	0.26	0.04	15	0.28	0.05	16	0.28	0.04
w, mg	31	1.08	0.11	18	1.1	0.09	15	1.7	0.15	16	2.94	0.87
%	32	96.7		23	78.3		20	66.7		46	35.0	
Year	2016											
Day	May 30			June 12			June 29			July 26		
	n	M	SE	n	M	SE	n	M	SE	N	M	SE
N	16	344.6	45.0	21	379.9	56.9	12	198.0	31.6	5	291.8	144.5
Wo, g	16	0.38	0.05	21	0.32	0.45	15	0.22	0.04	5	0.25	0.07
w, mg	16	1.18	0.1	21	1.18	0.12	12	2.4	0.6	5	0.9	0.4
%	16	5.8		23	8.0		20	40.0		19	74.0	

Note. Designations are same as in table 2.

Therefore, seasonal changes in reproductive characteristics impose certain restrictions on the comparative study. To obtain reliable comparative results it is necessary to focus the study on the period of maximum reproductive activity, when there are no females which already laid eggs, and the number of eggs in yastiks is maximum. Also, the analysis should be conducted in a population in which there are relatively equal shares of diploids and triploids. In this case, the analysis can be carried out only during the May–June populations in the Stugna River.

Comparison of reproductive characteristics of spined loaches in different rivers. Despite the apparent seasonal dynamics of the characteristics and some difference in time of sampling, the reproductive characteristics of spined loaches of different populations are clearly not the same (table 4). Female fishes of Stugna River population are evidently more fecund, as seen by the size of ovaries and number of eggs. The calculated egg size for this population is, inversely, smaller than that of Oster River population. The reason of unequal fecundity of spined loaches in different rivers can be both environmental and genetic factors. Comparing the size-and weight characteristics has shown that the length and weight of body of Oster River spined loaches are significantly lesser than that of fishes of Stugna River (table 5). The reasons may lie either in high density of this species' population or insufficient trophic base due to low productivity of Oster River ecosystems. In any case, unequal size of specimens in different rivers may also induce different fecundities. Still in this case the genetic factors cannot be eliminated. For Oster River population is almost fully polyploid while that of Stugna River is mostly diploid. Here it is necessary to consider the fact that egg size in spined loaches of the Oster River is almost twice larger than that of fishes of the Stugna River, certainly influencing fecundity.

Table 4. Comparison of reproductive characteristics of female spined loaches in populations of different river systems

River	Characteristics	2015			2016			For 2 seasons	
		n	M	SE	n	M	SE	M	SE
Stugna	N	47	797.4	66	30	898.1	95.3	835.8	55.4
	Wo, g	47	0.50	0.04	33	0.41 ±	0.04	0.45	0.03
	w, mg	47	0.8	0.08	30	0.79	0.19	0.79	0.09
Oster	N	80	398.4	47.5	57	320.2	27.1	365.8	30.0
	Wo, g	87	0.37	0.03	58	0.33	0.03	0.35	0.03
	w, mg	80	1.6	0.20	57	1.44	0.15	1.52	0.13

Note. Designations are same as in table 2.

Table 5. Comparison of size and weight characteristics of spined loaches in populations of different river basins

River	Characteristics	2015			2016		
		n	M	SE	n	M	SE
Stugna	L, mm	57	108.8	1.5	64	106.3	1.98
	l, mm	57	95.7	1.4	64	93.8	1.8
	W, g	51	5.9	0.21	64	5.54	0.22
Oster	L, mm	164	84.2	1.1	78	93.8	0.9
	l, mm	164	74.0	1.0	78	82.6	0.8
	W, g	164	2.81	0.1	78	3.4	0.08

Note. Designations are same as in table 2.

Comparison of reproductive characteristics of diploid and polyploid spined loaches. The obtained data allow comparing biological and reproductive characteristics of diploid and polyploid spined loaches sampled in Stugna River in May and June. One-way ANOWA has shown that all studied characteristics are significantly influenced by ploidy factor (table 6), and with the highest degree of significance this relates to such features as the number of eggs in the yastiks and their sizes.

Comparison of average values of mentioned parameters reveals the lack of linear relationship between size and weight characteristics and the level of ploidy (table 6). Tetraploids have maximum values size and weight, triploids have the minimum values, and diploids are of medium values. By productivity characteristics, there is a more linear relationship, associated with decreasing numbers of eggs (fig. 1) and ovary size (fig. 2), and increasing egg size together with ploidy (fig. 3). The latter relationship is quite understandable since the cell size in polyploid organisms usually exceeds that in diploids, as is the case with erythrocytes of spined loaches. Quite possibly the increased size of eggs

Table 6. Biological and reproductive characteristics of diploid (2n), triploid (3n) and tetraploid (4n) spined loaches of the Stugna river in May–June and the influence of ploidy on their variability

Characteristics	2n		3n		4n		F	P
	n = 29		n = 12		n = 5			
	M	SE	M	SE	M	SE		
L, mm	111.8	1.14	106.5	2.7	121.2	4.42	4.5	0.015
l, mm	98.3	1.05	94.1	2.48	107.8	4.2	4.9	0.011
W, g	6.41	0.16	5.38	0.37	7.64	0.71	7.3	0.001
Wo, g	0.55	0.03	0.50	0.05	0.25	0.06	5.2	0.008
N	1022.4	62.2	611.8	63.9	179.2	68.6	15.0	0.0001
w, mg	0.57	0.024	0.88	0.06	2.02	0.5	35.3	0.0001

Note. Designations are same as in table 2.

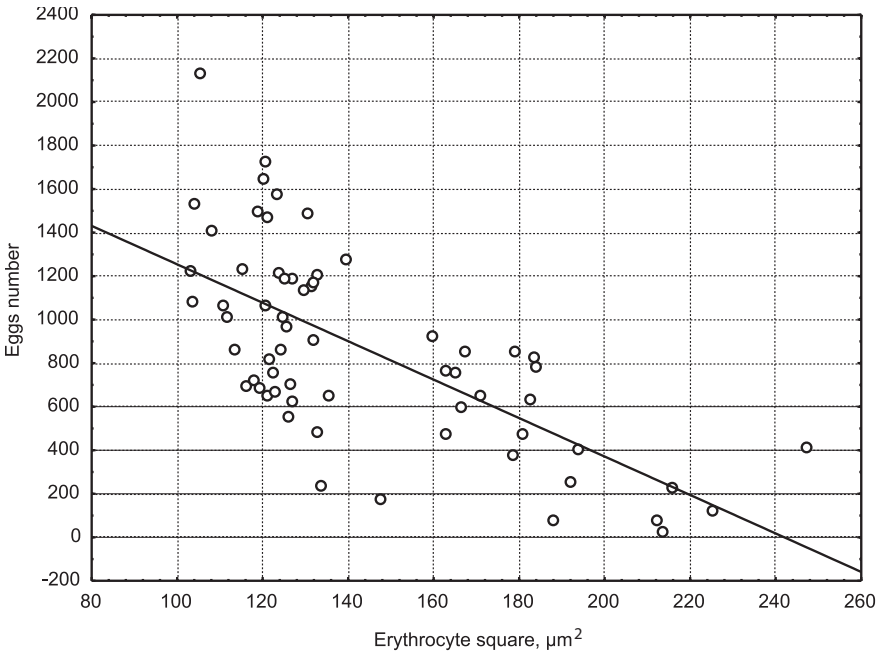

Fig. 1. Eggs number variation depend on erythrocytes area size (μm²) of spined loaches of Stugna River with different ploidy levels.

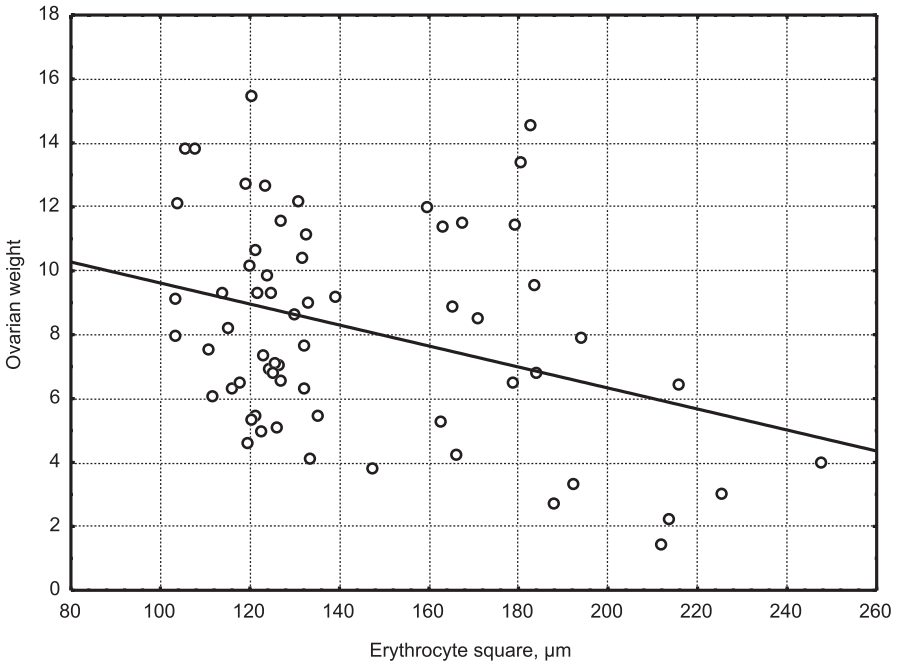

Fig. 2. Ovarium weight variation depend on erythrocytes area size (mμ) of spined loaches of Stugna River with different ploidy levels.

causes their reduced numbers in triploids. Whereas the sharp decline in fecundity in tetraploid specimens contrasting with their considerable size and weight may be indicative of the truly disturbed gametogenesis, resulting in underdevelopment of genitals and very low fecundity.

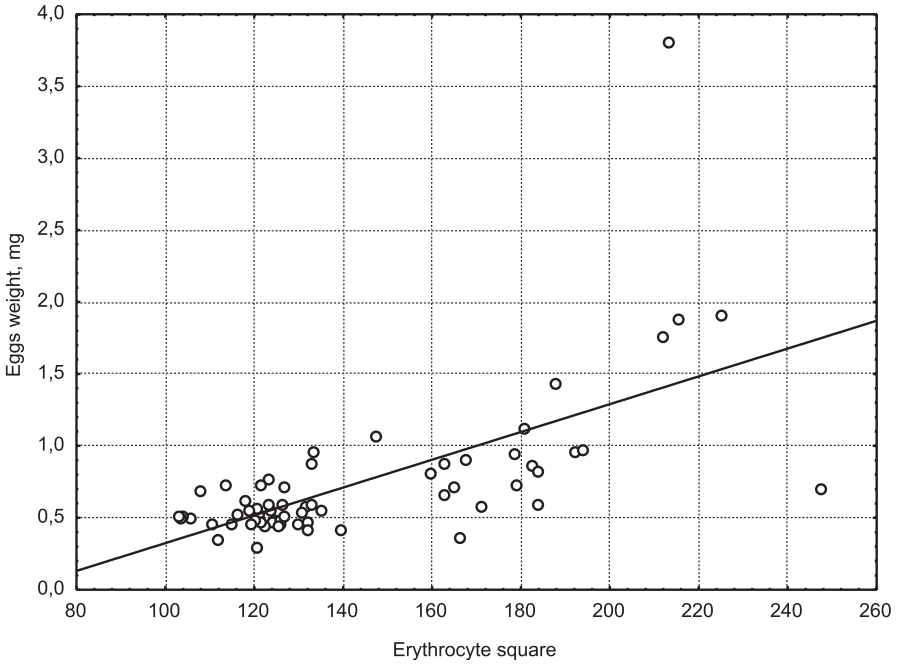

Fig. 3. Eggs weight (mg) variation depend on erythrocytes area size (mμ) of spined loaches of Stugna River with different ploidy levels.

Discussion of results

Therefore, the obtained results prove that polyploid spined loaches demonstrate reduced individual fecundity compared to diploids. Two mechanisms may cause this. First of all, the increasing egg size associated with amplified genetic material of polyploids and subsequent enlargement of the nucleus and cytoplasm, and disturbed gametogenesis. It can be safely assumed that triploids are limited by the former fecundity reducing mechanism. It follows from the fact that ovaries of diploids and triploids are similar, and the almost doubled egg size is proportional to the drop in fecundity. In other words this means that in the case of similar size ovaries the number of eggs would be greater the smaller they are. As for tetraploids, not only their absolute and relative weights of the ovaries rapidly decrease but their fecundity falls in a much greater degree than their egg sizes increase. This suggests that in the case of tetraploidy the leading role in reduction of fecundity is played by disorders of gametogenesis.

Concerning the question of whether the high productivity of polyploids is one of the causes of their expansion, the answer is not definitely negative. Although the diploid females are more fertile than the triploids, the overall reproductive potential of polyploids is higher than in diploids. In other words, 100 polyploid specimens would theoretically produce more offspring than 100 diploids. The reason for this is that polyploids are always female, while there are male and female diploids in equal proportions. As a result, higher numbers of potential offspring despite somewhat decreased fecundity are characteristic of polyploids.

Besides the reason for increasing numbers of polyploid spined loaches in Ukrainian water bodies also must be searched for among environmental factors. It is known that since 1960s, almost all Ukrainian plain's rivers have become regulated which has led to significant changes in aquatic ecosystems, particularly the changes in aquatic higher plant vegetation and chemical content of the rivers, also significant changes in numbers and ranges of freshwater fishes (Mezhzherin, 2008). These changes of environmental conditions must have been undoubtedly negative for the reproduction of aborigine diploid female loaches but possibly neutral for the expanding polyploids. Although this is a simplified view, since studies of spined loaches of the Desna River (the only unregulated large river of Ukrainian plains) have shown that there also polyploid forms prevail (Mezhzherin, Chudakorova, 2002). It is possible that there is a complex of yet not fully understood factors at work too.

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