ARTIFICIAL PROPAGATION AND REVEALED REPRODUCTION FEATURES OF WEATHERFISH (*MISGURNUS FOSSILIS*)

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VEŠTAŠKO RAZMNOŽAVANJE I REPRODUKTIVNE OSOBINE ČIKOVA (*MISGURNUS FOSSILIS*)

Apstrakt

U našem istraživanju je dvanaest ženki i osam mužjaka čikova veštački reprodukovano pre sezone mresta. Riba je uneta u laboratorijske tankove u rano proleće i ženke su tretirane sa 10 mg/kg telesne težine sa CP (ekstrakt hipofize šarana), dok su mužjaci hipofizirani sa 5mg/kg telesne težine radi izazivanja ovulacije i spermijacije. Ženke su ovulirale u narednih 18 do 24 časa i posle istiskivanja jaja su bila oplođena. Vrednosti pseudogonadosomatskog indeksa PSGI kod 4 ženke veoma su varirale (3.6 – 22.2%), stopa oplođenja je varirala od 30.34 do 93.81 % posle 24 časa od oplođenja. Tri dana po oplođenju larve su se izvalile (14.84-91.8%) i započele sa prvom egzogenom hranom šestog dana. Čikov se može razmnožavati kao i šaranske vrste u mrestilištima, jedina poteškoća je mala količina gameta. Veštačko razmnožavanje i uzgoj larvi može da pomogne u značajnom jačanju populacija, tako da bi bila moguća repopulacija već redukovanih populacija i stvaranje novih staništa koja odgovaraju ovoj vrsti. Vijabilne larve iz interspecijske hibridizacije su se izlegle i na osnovu njihove morfologije mlađ nije ličila na hibride, što bi moglo da ukaže na sposobnost aseksualnog razmnožavanja. Genetička analiza nije pokazala genom mužjaka kod mlađi. F1R1 potomci su bili 50% tetraploidi (4n=100)i 50 % heksaploidi (6n=150). Ovo je prvi rezultat stvaranja heksaploida (broj hromozoma 150) čikova u laboratorijskim uslovima.

Ključne reči: interspecijska hibridizacija, Misgurnus fossilis, Carassius carassius, ploidija
Keywords: inter-specific hybridisation, Misgurnus fossilis, Carassius carassius, ploidy

INTRODUCTION

Abundance of weatherfish (*Misgurnus fossilis*) has been decreasing from the last century mainly due to habitat destruction. It is categorized as „vulnerable" on the IUCN Red List. Systematic stockings of weatherfish into adjacent streams, canals and still waters might help to develop self-sustaining populations of *M. fossilis* in places where the species disappeared or occurs only sparsely. Artificial propagation and rearing of the larvae may help in strengthening population considerably, thus re-population of decreased stocks and creating new habitats – suitable for demand of the species – shall be possible. Our aim was to reveal its reproduction biology and improve effectiveness of its rearing under laboratory conditions.

MATERIAL AND METHODS

Spawning was induced by gradual increase of temperature in the tank from 10 to 20 °C for 6 days and females were injected with a single dose of 10 mg per kg of body weight of dry carp pituitary extract (CP) 18-24 hours before stripping, males were injected with a single dose of 5 mg per kg of body weight of CP. Females were anaesthetized in a solution of clove oil *(Syzygium aromaticum,* L. 1 aliquot per 1 L of water), then they were removed from the water onto wet towels and their genital aperture was dried with dry paper towels. Fertilisation tests of *M. fossilis* were carried out by using the dry fertilisation method. The testes were surgically removed and sperm was obtained by squeezing the organs through cheesecloth. This required sacrificing the males but enhanced sperm yield. The freshly stripped eggs were mixed with testicular sperm before freshwater was added to them. Eggs were incubated in 2 L plastic tanks and the larvae were reared in recirculation systems and grown exclusively on live food (*Artemia, Chironomus, Tubifex*) for 3 months.

In order to uncover the polyploidization and special asexual reproduction abilities of the species, stripped egg batches of weatherfish were fertilized by Crucian carp sperm (*Carassius carassius*) under laboratory conditions. Two females from this crossing were reared until mature stage (one year old fish) and propagated with *M. fossilis* males. After the propagation the females and males including *C. carassius* were investigated by using SCoT genetic analysis. *C. carassius* (male) genom were looked for offspring. The ploidy of embryos was determined using chromosome preparation and staining.

RESULTS AND DISCUSSION

The summarised result in connection with propagation in Table 1. can be seen.

Table 1. Summarised results of M. fossilis propagation * female originated intraspecific progation (M. fossilis × C. carassius).

No of females	Females body weight (g)	Stripped egg (g)	PGSI (%)	Fertilisation (%)	Hatching (%)
1	20.38	3.71	18.2	66.38	84.1
2	13.66	1.48	10.8	51.09	15.2
3	11.07	1	9.0	50.13	91.8
4*	11.57	0.42	3.6	30.34	14.81
5	66.99	12.59	18.8	93.13	72.8
6	61.39	11.36	18.5	93.81	71.5
7	70.45	3.21	4.6	52.94	51.5
8	28.1	6.228	22.2	81.02	70.4
9	25.77	2.485	9.6	53.61	53.1
10	25.30	1.804	7.1	64.19	51.7
11	20.29	1.164	5.7	90.62	48.8
12	19.45	2.116	10.9	92.15	33.3
mean±SD	**31.2±21.9**	**4.0±4**	**11.6±6.3**	**68.3±21.4**	**54.9±24.8**

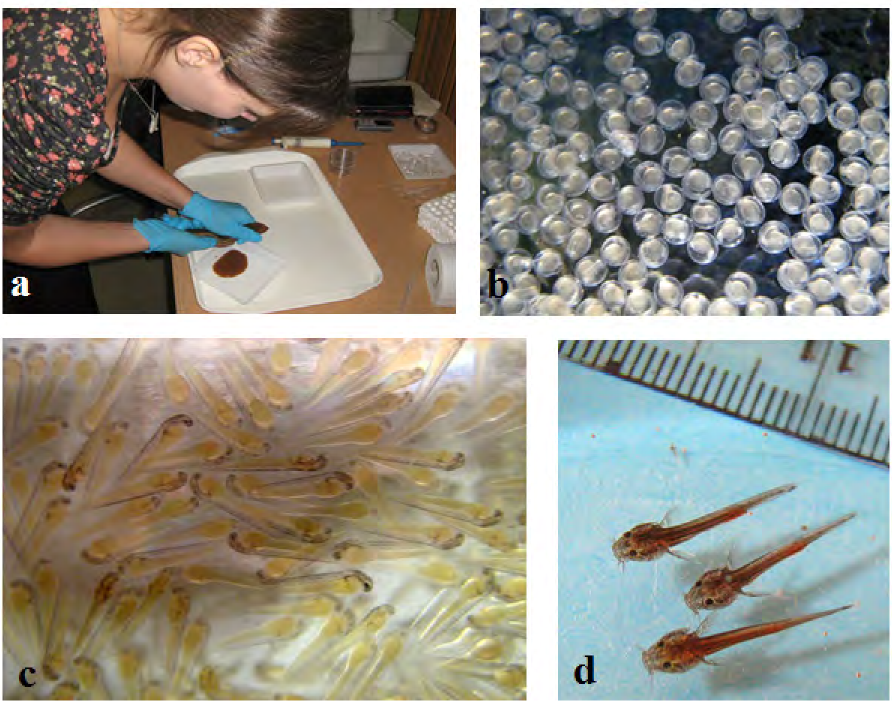

Figure 1. a: Egg stripping, b: Developing egg on the 2nd day, c: Embryos on the 1st day, d: 14 days old weatherfish.

Several reproduction parameters (latency time, fertilisation rate, hatching rate, etc.) and developmental factors were investigated and our results were similar which was described in literature (Drozd et al. 2009, 2010, 2011; Demény et al. 2009).

Reared, 3 month old weatherfish offspring provide a good stock for introduction to their original habitat or nature protected areas under optimal conditions (Table 2).

Table 2. Summarised data about the introduction (*Model Area for Hungarian protected and threatened marsh fish species).

Site	Date	Number of individuals	Spawning time	Introduced fish
Pusztaszer	04.09.2011	150	03. 2011	7-8 cm, 2-4 g
Szada, Illés-pond*, no. VI.	10.14.2011	20	03. 2011	8-10 cm, 3-5 g
Szada, Illés-pond*, no. II.	06.06.2012	200	05. 2012	1-2 cm
Szada, Illés-pond*, no. III.	06.06.2012	200	05. 2012	1-2 cm

From inter-specific hybridisation three larvae hatched, one of them died after two days, but two females were managed to rear to maturation phase. According to their morphology the weatherfish did not seem to be a hybrid that may refer to its ability to propagate asexually. The genetic analysis did not show male genom in the juveniles. One of them were managed to propagate and was fertilised with *M. fossilis* sperm. The results of chromosome preparation suggested F1R1 offspring were 50 % tetraploid (4n=100) and 50 % hexaploid (6n=150). According to result of Drozd et al. (2010), who investigated the ploidy level of wild *M. fossilis* in total, 19 triploids, 20 intermediate aneuploids and 77 tetraploids were recognized in a 1:1:4 ratio regardless of sex. The sex ratio of triploids and tetraploids was 1:1 and differed from that of intermediate aneuploids (3:1 for males). Our results showed to create viable hexaploid (150 chromosome number) *M. fossilis* under laboratory conditions.

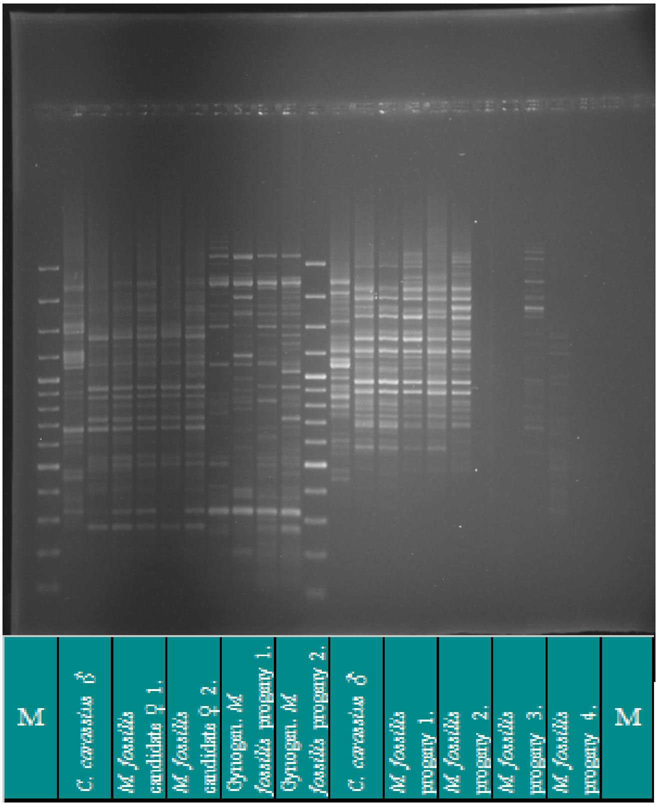

Figure 2. DNA pattern of broodstock (M. fossilis and C. carassius) and their offspring applying primer „SCoT 12".

CONCLUSIONS

Weatherfish can be propagated with the same method as Cyprinus-like species in hatchery, the only difficulty is the small amount of sperm.

In contrast to literature data about the ploidy level of wild *M. fossilis* viable hexaploid (n=150) fish could be created by using intra- and after interspecific propagation. Our final aim is to determine the ploidy and capability of hybridisation of both the maternal fish individuals and their offspring gained via sexual and asexual propagation. Flow cytometric investigations are in progress, that may make possible to reveal the chromosome number of all specimens in rearing including the grown offspring.

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