Aquacultura Hungarica (Szarvas), 1984. Vol. IV. pp. 33–38

56688

PRODUCTION OF STERILE HYBRID GRASS CARP (*CTENOPHARYNGODON IDELLA* VAL. X *ARISTICHTHYS NOBILIS* RICH.) FOR WEED CONTROL

Zoltán Krasznai[1], Teréz Márián[1], László Buris[2] and Ferenc Ditrói[2]

[1]*Fisheries Research Institute, H–5541 Szarvas, and*
[2]*Medical School of Debrecen, Debrecen, Hungary*

ABSTRACT

Triploid hybrids of female grass carp (*Ctenopharyngodon idella* Val.) and male bighead (*Aristichthys nobilis* Rich.) were produced, using artificial propagation. The triploid character was proved by karyological analyses. Morphological analysis was carried out on second summer hybrids. The scale form, the number of vertebrae and pharyngeal teeth can be used for distinguishing the hybrid from the parental species. The sterility of the hybrid was examined, and the results of the histological analyses of gonads as well as the results of sex hormone analyses are presented. The hybrid can be used for the control of the aquatic vegetation without the risk of overpopulation.

РЕЗЮМЕ

Авторы получили триплоидный гибрид со скрещиванием амура (*Ctenopharyngodon idella* Val.) и пестрого толстолобика (*Aristichthys nobilis* Rich.) Триплоидный характер гибрида доказали кариологическими анализами. Морфологические наблюдения провели на рыбах двухлетнего возраста. Гибриды отличаются от родителей по формуле чешуи, формуле глоточных зубов и количестве позвонков и лучей грудного плавника. Исследовали и стерильность гибрида. Сообщают о гистологических исследований гонадов и о результатах анализа полового гормона. Гибрид плодотворно можно использовать как биологическое истребительное средство без риска перенаселения.

KIVONAT

A szerzők triploid hibridet állitottak elő amur (*Ctenopharyngodon idella* Val.) és pettyes busa (*Aristichthys nobilis* Rich.) keresztezésével. A hibrid triploid jellegét kariológiai vizsgálatokkal bizonyitották. A morfológiai megfigyeléseket másodnyaras egyedeken végezték. A hibrideket a szülőktől a pikkelyképlet, a mellúszók sugarainak és a csigolyák száma, valamint a garatfogak képlete szerint lehet megkülönböztetni. Vizsgálták a hibrid sterilitását is. Közlik a gonádok szövettani vizsgálatának, valamint a szex-hormonok analizisének eredményeit.

A hibrid jól használható biológiai növényirtásra a túlnépesedés kockázata nélkül.

INTRODUCTION

The development of agriculture requires the use of more and more fertilizer, some of which is washed into natural waters and irrigation canals. Accumulation of these nutrients can cause excessive weed growth. At present chemical and mechanical methods are being applied for controlling aquatic vegetation. These techniques, however, are labour and cost consuming, do not completely solve the problem, and they can pollute the environment. For the biological control of aquatic plants grass carp (*Ctenopharyngodon idella* Val.) has been increasingly used in East Germany *(Janichen 1973)*, the Soviet Union *(Aliev 1976)*, and the Netherlands (van Zon 1977). In many countries this method is not allowed because of the danger of possible reproduction and naturalization of the species, following the introduction.

In 1975 a new triploid sterile hybrid of grass carp and bighead (*Aristichthys nobilis* Rich.) was developed in Hungary *(Márián and Krasznai 1978)* which can be used for the control of aquatic vegetation without the risk of unwanted overpopulation. It is cheaper, more effective and more acceptable in viewpoint of environmental protection.

The object of this paper is to describe the morphology and karyology of the hybrid and the results of its sex hormone and gonad histological analyses.

MATERIAL AND METHODS

The first crossbreeding of grass carp and bighead carp was carried out in 1975 *(Márián and Krasznai 1978)*. Induced breeding was used with the well known techniques described by several authors *(Aliev 1961, Vinogradov 1967)* and developed further by our own research team. Thirty-eight percent of the fertilized eggs developed to normal swim-up fry suitable for stocking, and they were stocked in specially prepared nursery ponds (500 m^2), five days after hatching, for a 30-day nursing period. Later on, they were raised in polyculture in somewhat bigger experimental ponds.

Morphological investigations have been carried out by using two-summer old hybrids with bodyweight of 250 to 300 g.

Fish of 20–80 g weight, propagated and reared at our Institute were used for karyological research, using the technique described in our previous paper *(Márián and Krasznai 1978)*. In the course of the microscopic examination of the chromosome sets, 50 cells were counted for each species. The diploid chromosome numbers were determined on the basis of the mean value.

Sex hormone investigation was carried out on five-year-old fish. The oestriol (pg/ml) and the testosterone hormone level (pg/ml) were determined by using the standard human RIA kit and procedure of the Radiochemical Center, England. Five-year-old hybrids, grass carps and bighead were used for histological analyses. Fish were decapitated and their gonads placed into neutral formalin. Paraffin sections were cut 7 μm and stained with haematoxylin and eosin *(Gurr 1962)*.

RESULTS

Karyological patterns. The most important karyological data, characteristic for grass carp, bighead and their hybrids are summarized in *Table I.* The diploid chromosome number of both parental species is 48, and the chromosomes form homogolous pairs. The morphological distribution of the karyotypes of the species is identical, and the number of chromosome arms (NF value) is also equal. Comparing the chromosome number of the hybrid (2n=72) with the diploid numbers of the parental lines, the hybrid is triploid. The triploid nature of the F_1 hybrid is also shown by the morphological distribution of the karyotype.

Karyological Chara

Species	2n
Grass carp	48
Bighead	48
F^2 hybrid	72

Morphological the back is darker

The scale form

The number of ro cies from the F_1 h

The body of the F_1 but the general im and there is no keel but a vertical line The finform of the

The number of r parental species (G The most common

But the following a

The pattern of the

The number of vert carp. Various indic body, these are sho

Sex hormone patt are summarized in 7 higher than that of In 5 cases from the the same, whilst in 3

Table I.

Karyological Characteristics of Grass Carp, Bighead and F_1 Hybrid

Species	2n	Meta-centric	Submeta-centric	Subtelo-centric	Acro-centric	NF
Grass carp	48	10	8	—	6	84
Bighead	48	10	8	—	6	84
F_1 hybrid	72	15	12	—	9	126

Morphological pattern of the F_1 hybrid. The dominant colour of the F_1 hybrid is green, the back is darker, the sides are lighter and the abdomen is yellowish-green or golden.

The scale form is: lateral line $52\frac{10-12}{9}54$

The number of rows of scales is an important feature in distinguishing the parental species from the F_1 hybrid.

Grass carp	Bighead
$42\frac{6-7}{5}45$	$114\frac{28-32}{23}120$

The body of the F_1 hybrid is somewhat flattened, the back is as broad as that of the bighead, but the general impression is very similar to that of the grass carp. Its abdomen is rounded and there is no keel to it. The form of the F_1 hybrid mouth is similar to that of the grass carp, but a vertical line drawn from the front of the eye does not reach the corner of the mouth.

The finform of the F_1 hybrid is: dorsal fin: iii 6–7
caudal fin: 24 (25)
anal fin: iii 8–10
abdominal fin: i—8
pectoral fin: i—15–17

The number of rays of the pectoral fin can also be used to distinguish the hybrid from the parental species (Grass carp: i—18; Bighead: i—18).

The most common formulae of pharyngeal teeth of the hybrid are: 4.1–1.4
5.1–1.3

But the following are also found: 5.1–0.4 5.1–2.5
5.1–1.5 4.1–2.5
4.1–1.4 3.1–1.4

The pattern of the pharyngeal teeth of the grass carp is: 2.5–4.2
2.4–4.2
2.4–5.2
1.4–5.2

The number of vertebrae in the hybrid is 38–39 compared with 42–43 vertebrae in the grass carp. Various indices were used to describe the characteristic ratios of different parts of the body, these are shown in *Table II.*

Sex hormone pattern. The results of hormone level determinations from the blood serum are summarized in *Table III.* In female grass carp or bigheads the level of oestriol was much higher than that of testosterone, whilst in male grass carp or bigheads the reverse was true. In 5 cases from the examined 9 hybrids the levels of oestriol and testosterone were nearly the same, whilst in 3 cases high testosterone and low oestriol levels were obtained.

Table II

Characteristic Body Indices of Grass Carp, Bighead and Grass Carp×Bighead F_1 Hybrids

	Grass carp index	%	Bighead index	%	F_1 hybrid idex	%
Profile index TL/HB	4.714	21.21	4.224	23.67	4.884	20.48
Caudal index TL/LT	5.419	18.45	4.486	22.29	4.605	21.17
Head index TL/LH	4.935	20.26	3.697	27.04	4.277	23.38
Width index TL/WB	6.761	14.78	8.394	11.91	8.024	12.46
Height index HB/WB	1.434	69.72	1.987	50.32	1.643	60.84

TL: total length; HB: height of body; WB: width of body; LT: length of tail; LH: length of head.

Table III.

The E (Oestriol) and Testosterone Hormone Levels (pg/ml) in the Blood of Grass carp, Bighead and the F_1 Hybrid

No. of sample	Name of fish	E (oestriol) hormone, pg/ml	Testosterone hormone, pg/ml	Presumable sex
1	GC×BH	111.0	178.0	U
2	GC×BH	68.7	90.0	U
3	GC×BH	126.4	157.3	U
4	GC×BH	140.2	107.0	U
5	GC×BH	123.2	202.5	U
6	GC×BH	110.8	296.3	♂
7	GC×BH	92.3	352.2	♂
8	GC×BH	76.5	312.8	♂
9	GC×BH	70.3	304.0	♂
10	GC	845.3	242.0	♀
11	BH	672.1	291.3	♀
12	GC	125.8	813.0	♂
13	GC	266.4	867.7	♂
14	GC	244.2	504.0	♂
15	GC	88.0	325.5	♂
16	BH	85.8	294.0	♂
17	BH	56.2	252.0	♂
18	BH	56.2	630.0	♂

GC = grass carp
BH = bighead
GC × BH = F_1 hybrid

Histological pattern of gonads. The gonads of the examined five-year old grass carp and bighead females and males were normally developed. In the case of the hybrid only abnormal or less developed gonads were found. Oocytes from the triploid hybrid ovaries were abnormal, compared with the ovaries of the diploid parents. In the ovaries of some hybrids insular testicular tissue was found in a mass of indifferentiated cells.

DISCUSSION

By crossing the grass carp and bighead viable triploid hybrids were produced which have some advantageous characteristics. The triploid character of the hybrid was proven by karyol-

ogical analyses. Me
USA and confirmed
lebrates, especially
Uyeno 1972, Allen &
produced by interspe
Triploidy has been
Lincoln et al. 1974,
of the retention of
sterile because they
Induced polyploic
Stanley 1978). In trip
(Platichthys flesus)
hybrids of *(Poecilio*
1968).
Bakos et al. *(1978*
back-crosses of thes
molitrix Val.) yielde
development would
Suzuki *(1963)* report
between *Pseudogobio*
The gonads of the
nads of the hybrids
same age, and defo
hybrids. The histolog
of triploid fish were
some hybrids "island
Lincoln *(1981)* also
tained in the oestriol
hybrids was less thar
testosterone were ob
In the USA hybric
sible role in weed co
underyearling hybrid
further studies are re
it is already used for v

REFERENCES

Allen S. K., Stanley J. G. 19
107 : 473–478.
Alies D. S. 1961. Amur i lok
Aliev D. S. 1976. The role o
ment of water. J. Ichthy
Bakos J., Krasznai Z., Mari
Aquacult. Hung. 1 : 51–
Cupana F., Calaudella S., G
x Salvelinus fontinalis (
Cassani J. R. 1984. Feeding
(Hypophthalmichthys n
Cimino C. M. 1974. The nuc
the evolution of unisexu
Cuellar O., Uyeno T. 1972.
Gervai J., Peter S., Nagy A
17 : 667–671.
Gurr E. 1962. Staining anima
Janichen H. 1973. Die biolog
Lincoln R. F., Aulstad D., G
cold shocks. Aquacultur

ogical analyses. Melvin and Biggers *(1980)* reported the production of this hybrid in the USA and confirmed the triploid character. Triploids are generally more viable in lower vertebrates, especially in fishes. There are some naturally occurring triploid fish *(Cuellar and Uyeno 1972, Allen and Stanley 1978, Cimino 1974, Thorgard and Gall 1979)*, and some are produced by interspecific hybridization *(Capana et al. 1974, Vasiljev et al. 1975, Purdom 1972)*. Triploidy has been experimentally induced using cold or heat shock *(Wolters et al. 1981, Lincoln et al. 1974, Gervai et al. 1980)*. It is believed that in these cases triploidy is the result of the retention of the second polar body by the ovum; such triploids are expected to be sterile because they would produce aneuploid gametes *(Melvil and Biggers 1980)*.

Induced polyploidy may be a method for producing permanently sterile fish *(Allen and Stanley 1978)*. In triploids of the hybrid between plaice (*Pleuronectes platessa* L.) and flounder *(Platichthys fleus)* abnormal gametogenesis was observed *(Purdom 1972)*, and triploid hybrids of (*Poecilia formosa* L.) and *(Poecilia sphenops)* were sterile *(Schultz and Kallman 1968)*.

Bakos et al. *(1978)* reported that crosses between common carp and silver carp hybrids and back-crosses of these hybrids to parental species (*Cyprinus carpio* L. or *Hypophthalmichthys molitrix* Val.) yielded no fertilized eggs that developed beyond the plastodisc stage. Similar development would be expected of aneuploid gametes produced by the grass carp hybrid. Suzuki *(1963)* reported sterile males and hermaphrodite hybrids from the intergeneric cross between *Pseudogobio esocinus* and *Gnathopogon elongatus*.

The gonads of the five-year-old hybrids, grass carp and bighead were examined. The gonads of the hybrids were less developed in each case than that of the parental species of the same age, and deformities were found. In some cases hermaphroditism occurred in the hybrids. The histological analyses confirmed the visual examination. Oocytes from the ovaries of triploid fish were abnormal compared with the ovaries of the parents. In the ovaries of some hybrids "island located" testicular tissue was found in a mass of undifferentiated cells. Lincoln *(1981)* also reported abnormal ovaries in triploid flatfish. Similar results were obtained in the oestriol and testosterone hormone analyses. The testosterone level of the male hybrids was less than that of the grass carp males, and in cases similar levels of oestriol and testosterone were obtained, which suggested the hermaphrodite nature of these fish.

In the USA hybrid grass carp have been continuously produced since 1978 and their possible role in weed control assessed. Cassani *(1981)* examined the feeding behaviour of the underyearling hybrids and reported it to be similar tothat of the grass carp parent. Although further studies are required on food intake, food preference, and growth rate of the hybrid it is already used for weed control in a few of the western countries.

REFERENCES

Allen S. K., Stanley J. G. 1978. Reproductive sterility in polyploid brook trout, Salvelinus fontinalis. Trans. Am. Fish. Soc. 107 : 473–478.

Aliev D. S. 1961. Amur i tolsztolob v Turkmenii. Rübovodstvo i Rüboloistvo 22 : 14–16.

Aliev D. S. 1976. The role of phytophagous fishes in the reconstruction of commercial fish fauna and biological improvement of water. J. Ichthyol. 16 : 216–229.

Bakos J., Krasznai Z., Marian T. 1978. Cross-breeding experiments with carp, tench and Asian phytophagous Cyprinids. Aquacult. Hung. 1 : 51–57.

Capana E., Cataudella S., Centile de Fronza T. 1973. Some remarks on karyotype of an intergeneric hybrid, Salmo trutta x Salvelinus fontinalis (Pisces: Salmoniformes). Genetica 44 : 194–206.

Cassani J. R. 1981. Feeding behaviour of underyearling hybrids of grass Carp (Ctenopharyngodon idella) and highead (Hypophthalmichthys nobilis) on selected species of aquatic plants. J. Fish. Biol. 18 : 127–133.

Cimino C. M. 1974. The nuclear DNA content of diploid and triploid Poeciliopsis and other Poecilid fish with reference to the evolution of unisexual forms. Chromosome (Berl.) 47 : 297–307.

Cuellar O., Uyeno T. 1972. Triploidy in rainbow trout. Cytogenetics 11 : 508–515.

Gervai J., Péter S., Nagy A., Horváth L., Csányi V. 1980. Induced triploidy in carp. Cyprinus carpio L. J. Fish. Biol. 17 : 667–671.

Gurr E. 1962. Staining animal tissues: practical and theoretical. Leonard Hill Books, London.

Janichen H. 1973. Die biologische krautung. Institut f. Binnenfischerei Berlin-Friedrichshagen 44. (in German).

Lincoln R. F., Aulstad D., Grammeltvedt A. 1974. Attempted triploid induction in Atlantic salmon (Salmo salar) using cold shocks. Acuaculture 4 : 287–297.

Lincoln R. F., 1981. Sexual maturation in female triploid plaice Pleuronectes platessa, and plaice x flounder Platichthys flesus hybrids, J. Fish. Biol. 19 : 499–507.
Márián T., Krasznai Z. 1978. Karyological investigations on Ctenopharyngodon idella and Hypophthalmichthys nobilis and their cross breeding. Aquaculture Hungarica I : 44–50.
Melvin L. B., Diggers C. J. 1980. Karyological analysis of Ctenopharyngodon idella, Aristichthys nobilis and their F1hybrid. Trans. Am. Fish. Soc. 109 : 437–438.
Purdom C. E. 1972. Induced polyploidy in plaice (Pleuronectes platessa) and its hybrid with the flounder (Platichthys plesus). Heredity 29 : 11–24.
Schuhz R. J., Kallman D. 1968. Triploid hybrids between the all-female teleost Poecilia formosa and Poecilia sphenops Nature 219 : 280–282.
Suzuki R. 1963. Hybridization experiments in cyprinid fishes. II. Reciprocal crosses between Pseudogobio esocinus and Gnathopogon elongatus elongatus, Jap. J. Gen. 38 : 89–96.
Thorgard G. H., Gall G. A. E. 1979. Adult triploid in a rainbow trout family. Genetics 93 : 961–973.
Vasiljev V. P., Makeeva A. P., Rhabov I. N. 1975. O triploidii gibridov karpa sz drugimi predstaviteljami semejstva Cyprinidae. Genetika Tom. XI. : 49–56.
Vinogradov V. 1967. V rubepiromnike "Gorjacskij Kluc" Rubovodstvo i Rúholovstvo 10 : 4.
Wolters W. R., Libey G. S., Chrisman C. L. 1981. Induction of triploidy in channel catfish. Trans. Am. Fish. Soc. 110 : 310–312.
Van Zon J. C. J. 1977. Grass carp, Ctenopharyngodon idella Val. in Europe. Aquatic Botany 3 : 143–155.