Bulgarian Journal of Agricultural Science, 28 (Suppl. 1) 2022
Agricultural Academy

Application of carp pituitary and Nerestin in artificial reproduction of species of the family Cyprinidae

Berdibek Abilov[1,2*], Lyudmila Nikolova[3], Kuanish Isbekov[2], Gulzhan Kulmanova[1]

[1]*Kazakh National Agrarian University, Almaty, Kazakhstan*
[2]*Scientific and Production Center of Fisheries LLC, Almaty, Kazakhstan*
[3]*Agricultural University, Department of Animal Science, 4000 Plovdiv, Bulgaria*
[*]*Corresponding author:* b.i.abilov@mail.ru

Abstract

Abilov B., L. Nikolova, K. Isbekov & G. Kulmanova (2022). Application of carp pituitary and Nerestin in artificial reproduction of species of the family Cyprinidae. *Bulg. J. Agric. Sci.*, *28 (Supplement 1)*, 129–138

Some reproductive characteristics after application of carp pituitary and Nerestin in artificial reproduction of common carp, grass carp and silver carp from local populations in Kazakhstan were studied. Application of Nerestin-6 showed relatively low efficacy in all the studied fish species. Fecundity and egg hatchability of common carp, grass carp and silver carp were 73.2% and 32.5%; 72.5% and 43.2%; 75.3% and 41.2%, respectively. Nerestin-1B proved to be the most efficient in herbivorous fish. When used in grass carp and silver carp, egg fecundity and the percentage of hatched prelarvae was 92.5% and 53.0%; 88% and 50.2%, respectively. In common carp, the best results were obtained after the application of pituitary extract. Fecundity and hatchability of the eggs reached 92.5% and 54.5%, respectively.

Keywords: carp, grass carp, silver carp, artificial propagation, fecundity, egg fertility, prelarvae
Abbreviations: Cc – Common carp; Gc – Grass carp; Sc – Silver carp; BW – Body weight; L – Total length; SL – Standard length, Hl – Head length; H – Maximum body height; O – Maximum body girth; B – Maximum body width; CF – Fulton's coefficient $((BW/SL^3)*100)$; HBI – High-backed index (l/H); IBB – Broad-backed index ((B/SL)*100); IH – Hardness index (O/SL) × 100; AF – Absolute fecundity, eggs; RFg – Relative fecundity, eggs/g BW; RFcm – Relative fecundity, eggs/L cm; EFR – Egg fertility rates, %; HP – Hatched prelarvae, %.

Introduction

In recent years, aquaculture in Kazakhstan has been developing rapidly and about 18 thousand tonnes of commercial fish were produced in the period 2015 – 2019 (Anonymous, 2021). Pond fish culture is the most widespread in the country with the most promising farms specializing in rearing carp and herbivorous fish species (Alpejsov et al., 2015).

Two common carp subspecies are found in natural habitats in Kazakhstan (Gvozdev, 1988). Those are European carp (*Cyprinus carpio carpio* Svetovidov) and Aral carp (*Cyprinus carpio aralensis* Spitshakow). European carp is found in the Ural-Caspian and Embinska rivers. Aral carp is widespread in the ground waterbodies of Kazakhstan. The natural habitat of Aral carp includes the Aral Sea and the river systems of the Amu Darya, Zeravshan, Syrdarya, Sarysu and Shu rivers with their associated lakes (Kamyshlybash, Akchatau, Tilikol) and dams (Shardara, Bogen). Aral carp was introduced to other waterbodies (Assylbekova et al., 2018). Herbivorous fish species in Kazakhstan emerged as a result of a purposeful introduction in the middle of the last century (Assylbekova & Kulikov, 2016). Nowadays grass carp is widely spread in practically all major waterbodies in Central Asia and Kazakhstan. Silver carp is found in the northern regions of Kazakhstan in the basins of the Irtysh, Tobol and Esil rivers, and in the south in the basins of the Syr Darya and Ili rivers.

Woynarovich et al. (2010) underlined that carp pond polyculture is the most widely practiced fish production system in different regions in Central and Eastern Europe, the Caucasus and Central Asia. Introduction of carp and herbivorous fish rearing in polyculture is the most significant factor in increasing the efficiency of warm-water fish farming (Grozev et al., 1999). Representatives of Cyprinidae family are widely represented in world aquaculture. According to the latest data, the relative share of grass carp is 10.5%, silver carp – 8.8% and common carp – 7.7% out of the total finfish aquaculture worldwide (FAO, 2020). In recent years, more than 200 million fry of carp, grass carp, silver carp, etc. are produced annually from reproductive hatcheries in Kazakhstan (Kulikov & Assylbekova, 2020).

Development and implementation of effective reproductive technologies are the basis for increasing the efficiency of aquaculture (Weber, 2011; Weber & Lee, 2014). Hormonal treatment of the reproduced individuals is used in artificial propagation of fish. A number of synthetic preparations are widely used along with fish pituitary (Zohar & Mylonas, 2001; Brzuska, 2006; Jha & Neupane, 2019). In a study of hormonal stimulation in common carp, grass carp and silver carp, Farag et al. (2017) found that the use of appropriate types of hormonal preparations can increase the effectiveness of controlled reproduction.

In a number of comparative studies, better results on some reproductive characteristics were obtained using pituitary extract (Farag et al., 2017; Lyubomirova et al., 2020, etc.). In a study of the effect of carp pituitary and Ovaprim in artificial reproduction of *Colossoma macropomum*, Acuña & Rangel (2009) found that a significantly higher number of fish eggs were obtained using the analogue of the GnRH, but better fecundity and hatching rates were achieved after treatment with pituitary extract. In a comparative study of artificial propagation of *Perca fluviatilis* with carp pituitary and Nerestin-6A, Barbacariu et al. (2018) established better results after treatment with Nerestin. Nerestin has been developed in different modifications for use in different fish species. It has many advantages over pituitary (Dima et al., 2009) and it is used in carp (Radu et al., 2018) and herbivorous fish (Popescu et al., 2018).

The aim of the study was to make a comparative study of the application of carp pituitary and Nerestin in the artificial reproduction of common carp, grass carp and silver carp from local populations in Kazakhstan.

Materials and Methods

The study was carried out in 2020 in a fish farm pond and in a fish hatchery of the Kapshagai spawning and breeding farm, located in the Enbekshikazakh area of the Almaty region.

The subject of the study were: breeders (broodstock), eggs and hatched larvae of:

Common carp (*Cyprinus carpio*)
Grass carp (*Ctenopharyngodon idella*)
Silver carp (*Hypophthalmichthys molitrix*).

An experimental design was developed for the needs of the study, including primary complex assessment of the breeding material, preparation of the individuals for reproduction, application of hormonal preparations, obtaining the sexual products (eggs and sperm), fertilization of the eggs, preparation and placing the eggs in hatcheries, egg incubation. Throughout the period of the experiment, the aquatic environment of the breeders, eggs and larvae was constantly monitored.

Primary complex assessment of the breeders and formation of experimental groups

In winter, the breeders were reared in special winter ponds at a stocking rate not exceeding 10 $t.ha^{-1}$. In spring, when the breeders were caught from the winter ponds, an assessment of the fishes was carried out.

Only healthy, non-traumatic male and female fishes of all the species were selected for the needs of the experiment. In addition, female fishes were provided as a reserve.

At the initial complex on-site inspection, the females from each species were divided into three groups. Female fishes with well-defined sexual characteristics were placed in the first group. Female fishes with less developed sexual characteristics were put in the second group. In female individuals of the second group, it was noted that the bellies were relatively harder on palpation. However, the analysis of their eggs showed that the nuclei in the oocytes were located in the animal pole, which indicated a good degree of maturity. Female fishes, which did not differ in appearance from the male fishes and those with poorly developed sexual characteristics, were placed in the third group and they were not used for reproduction. Fishes from the first group were used for the experiment.

One group of male fishes was formed from each species. For common carp, a total of 7 females aged 7 to 11 years and 3 males aged 7 to 9 years were used; for grass carp a total of 9 females aged 7 to 11 years and 3 males aged 6 to 9 years and for silver carp – 9 females 7 to 9 years old and 3 males 8 to 9 years of age. Groups for different hormonal treatment were formed from the initially selected female fishes from all the species.

The selected individuals were transported to the fish hatchery and placed in a special pond in individual cages. During transport and work, the specific issues for the welfare of the fishes were followed, requiring careful handling.

Conventional classical methods for artificial reproduction of thermophilic fish species were applied (Zalepuhin, 2009). The method used in the present experiment was developed and recommended by Pushchino research-and-production complex 'Aquaculture' (http://www.nerestin.ru) for application of the synthetic preparation 'Nerestin-1B', specially developed for herbivorous fish, and Nerestin-6, used as a universal preparation.

Two groups of female caps fishes were studied: females stimulated with Nerestin-6 (n = 4) and a control variant stimulated with pituitary extract (n = 3).

Three groups of female grass carp fishes were formed, stimulated with: 'Nerestin 1 B' (n = 3), 'Nerestin 6' (n = 3) and a control group stimulated with pituitary gland (n = 3), respectively.

Three groups of silver carp females were also formed stimulated with: Nerestin 1B (n = 3), Nerestin 6 (n = 3) and a control group stimulated with pituitary extract (n = 3). The male carp fishes (n = 3), grass carp (n = 3) and silver carp (n = 3) were stimulated only with pituitary extract. Table 1 presents the scheme of hormonal treatment of fishes.

The suspension for injection was prepared individually for each producer. Fishes were placed on a special table with a soft cover for receiving the hormonal injection. Hormonal treatment in female fishes was applied twice. The preliminary pituitary dose was 10% of the total amount of the preparation. The preliminary dose of both types of Nerestin was 20% of the total amount.

The interval between the first (preliminary) and the second (resolving) injection was 13 hours. The preliminary injection was given at 20:00 PM in the evening and the resolving at 9:00 AM on the next day.

All the male fishes used in the study were treated with pituitary extract. The injection was a single dose of 2 mg/kg body weight. The males were injected at the time of the second dose for the female fishes.

Aquaculture and biological data were reported and processed following generally accepted methods (Anonymous, 1986; Assylbekova et al., 2013, 2014; Kozlov, 1998; Ryzhkov, 1987).

After the breeders were transferred to the fish hatchery, daily control monitoring was performed. The general condition of the breeders was controlled: the dynamics of external characteristics – belly softness, swelling and redness of the genitals, egg development level.

The level of readiness of the female breeders was determined according to the location of the nucleus in the oocyte. For that purpose, samples were collected by biopsy with a special steel probe and they were analyzed with a microscope MBS – 10.

When sperm was obtained from all the male breeders, the volume of ejaculate was determined individually.

The absolute fecundity of female fish was established by the weight method. A sample of 500 mg was taken from the eggs of each female producer. In each sample, the eggs were counted using an MBS – 10 microscope, after which the total number of eggs was calculated.

Table 1. Applied doses of Nerestin and pituitary extract

Injections	Fish Species/Sex					
	Carp		Grass carp		Silver carp	
	Female	Male	Female	Male	Female	Male
Pituitary						
Preliminary, %	10	–	10	–	10	–
Resolving, %	90	100	90	100	90	100
Total, mg/kg BW*	3.3	2.0	4.5	1.5	4.5	1.5
Nerestin-6						
Preliminary, %	20	–	20	–	20	–
Resolving, %	80	–	80	–	80	–
Total, ml/kg BW*	0.5	–	0.5	–	0.5	–
Nerestin-1B						
Preliminary, %	–	–	20	–	20	–
Resolving, %	–	–	80	–	80	–
Total, ml/kg BW*	–	–	0.33	–	0.33	–

*BW – body weight

Fish hatchery equipment

The fish hatchery was equipped with all the necessary facilities for the artificial spawning of fish, providing water exchange of about 260 $l.min^{-1}$ and automatic maintenance of the optimal water temperature regime.

A pond with a depth of 1.7 m was equipped for keeping temporarily the content of the breeders in the fish hatcher. Six trays with dimensions 340 × 70 × 80 cm were also provided for the adult fishes and hatched larvae. Each tray was divided by nets into three parts.

The fish hatchery was equipped with 16 Weiss incubation systems and 15 Amur incubation systems.

The conditions provided for the breeders' contents, the regime in the hatcheries and in the prelarvae and larvae trays were monitored daily, analysing the water temperature, oxygen content and pH. A thermooximeter Mark – 302 was used for that purpose.

The breeders were provided with a constant optimal regime of the water environment (Table 2) and a water volume of 10-15 $l.min^{-1}$.

Water temperature was raised to the optimal value (22.1℃) within the first 24 hours after placing the breeders in the trays and the fishes stayed in the trays for up to 5 days.

Table 2. Characteristics of the hydrochemical regime in the pond and in the trays

Parameter	Data		
	Mean	Min	Max
Water temperature, °C	21.2	20.3	22.1
Dissolved oxygen, $mg.l^{-1}$	7.05	6.9	7.2
pH	8.05	7.8	8.3

Statistical data processing was done with working version 19 of SPSS program. The differences between the groups (the hormonal preparation used within the species and the species within the applied hormonal preparation) were determined by Fisher's LSD method.

Results and Discussion

A complex evaluation of all the fishes was carried out at the beginning of the experiment. Table 3 presents the characteristics of the male fishes of the different species. All individuals were well-developed, with optimal body weight for reproduction and in a good nutritional status.

The indicators characterizing the female fishes of the different species and groups are presented in Table 4.

The body weight of common carp representatives varied within the range of 3140 – 7860 g. It is not recommended to work with too large fish breeders as this makes it difficult to handle them in the fish hatcheries. The female carp representatives selected for the experiment were well-developed, with optimal body weight and in a good nutritional status. As in the male carp fishes, the Fulton's coefficient was high, which indicated the good rearing conditions provided for the studied individuals.

When working with female carp fishes, the main focus was on the condition of the belly and genitals. All fishes had well-developed external sexual characteristics. The bellies were soft, the genitals swollen and red.

After the resolving injection, the female fishes were constantly monitored. The observed change in behaviour (anxiety) was a sign of the fish readiness for reproduction. The eggs and sperm were harvested by manual stripping, gently

Table 3. Characteristics of male breeders of the different species

Indicators	Carp (n = 3)		Grass carp (n = 3)		Silver carp (n = 3)	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
BW, g	4740.0	1442	6315.0	731.2	6001.0	97.90
L, cm	626.7	53.46	790.0	45.82	770.0	26.46
SL, cm	535.0	47.69	693.3	46.46	676.7	20.82
Hl, cm	131.0	8.888	158.3	15.94	177.7	8.627
H, cm	151.7	14.74	149.7	20.23	173.3	0.577
O, cm	438.3	30.86	416.7	35.12	500.0	26.46
B, cm	71.67	3.511	91.00	9.643	111.7	2.889
CF	3.02	0.257	1.90	0.183	1.94	0.150
HBI	3.53	0.080	4.66	0.332	3.90	0.129
IBB	13.43	0.551	13.11	0.879	16.51	0.737
IH	0.82	0.037	0.60	0.019	0.74	0.021

Table 4. Characteristics of female breeders of the different species, mean ± SD

Indices	Common carp (n = 7)				Grass carp (n = 9)						Silver carp (n = 9)					
	Groups															
	Pituitary (n = 3)		Nerestin-6 (n = 4)		Pituitary (n = 3)		Nerestin-6 (n = 3)		Nerestin-1B (n = 3)		Pituitary (n = 3)		Nerestin-6 (n = 3)		Nerestin-1B (n = 3)	
	ΔX	SD	ΔX	SD	ΔX	SD	ΔX	SD	ΔX	SD	ΔX	SD	ΔX	SD	ΔX	SD
BW, g	6353.3	1308.64	4175.0	736.05	7212.0	1211.0	6513.0	977.0	7161.0	2027.0	5949.0	606.7	5991.0	912.7	6408.0	1511.0
L, cm	700.0	30.00	611.3	22.50	793.3	25.66	773.3	15.28	823.3	62.12	785.0	18.03	790.0	20.00	770.7	59.00
SL, cm	603.3	41.63	513.7	18.88	706.7	23.63	683.3	17.56	723.3	57.74	688.3	12.58	695.0	13.23	680.7	59.00
Hl, cm	141.7	4.51	133.5	6.56	166.0	5.29	157.3	0.57	164.3	18.01	179.0	8.88	182.3	6.80	180.3	13.79
H, cm	165.0	3.61	154.0	12.06	153.0	9.54	141.7	8.08	159.7	16.77	177.3	2.51	178.0	4.35	175.3	2.51
O, cm	503.0	18.00	443.0	39.40	456.7	30.55	416.7	47.26	415.0	44.44	516.7	32.15	500.0	17.32	500.0	30.00
B, cm	96.7	16.07	76.7	4.27	110.0	10.0	110.0	10.00	105.6	21.13	114.7	5.03	117.7	8.73	111.3	11.72
CF	2.8	0.09	3.1	0.26	2.0	0.19	2.0	0.18	1.8	0.06	1.8	0.08	1.7	0.16	2.0	0.09
HBI	3.7	0.19	3.4	0.18	4.6	0.14	4.8	0.15	4.5	0.11	3.8	0.02	3.9	0.10	3.8	0.28
IBB	15.9	1.52	14.9	0.30	15.6	0.93	16.1	1.33	14.5	1.69	16.6	0.63	16.9	1.56	16.3	0.66
IH	0.8	0.03	0.9	0.05	0.7	0.03	0.6	0.05	0.6	0.02	0.7	0.03	0.7	0.01	0.7	0.02

massaging the belly areas. 1.2 – 2.0 cm^3 of sperm were obtained. 3 – 4 g of hetero sperm was used per 1 kg of row for fertilization.

The eggs fertilization was carried out by the dry method. The eggs and sperm were gently mixed with bird feathers for 10 – 20 seconds, after which 100 – 150 ml of carp pond water was added, followed by stirring for 40 – 60 seconds.

Milk diluted with water in a ratio of 1:10 was used for de-adhesion of the fertilized carp eggs. De-adhesion was performed in Weiss type devices. The average time of de-adhesion was about an hour. De-adhesed eggs were put for incubation in Amur devices. The water exchange in Amur devices was maintained at 10 l/min for 1 million eggs. Immediately before hatching of the prelarvae, water exchange decreased and after the mass hatching, it increased again to 12 l/min.

During the incubation period and after hatching, water quality in the apparatus was constantly monitored. The oxygen content varied within the range of 7.1 – 8.2 $mg.l^{-1}$. Larvae growth was controlled with binoculars every day.

Five days after the beginning of egg incubation in the devices, when the larvae had already switched to exogenous feeding, they were transferred to ponds.

Table 5 presents the results about the reproductive characteristics of the female fishes from the groups with different hormonal treatment. The time of carp maturation in the different groups did not differ and it was 12 hours.

In the study by Farag et al. (2017) it was found that pituitary-stimulated carp fishes also matured in 12 h, while after the application of synthetic preparations the maturation time was longer – from 13 to 16 h. In the same study, the hatching rate of pituitary-treated carp was 50%, while the indicator was lower after treatment with other hormonal preparations. Lyubomirova et al. (2020) also obtained better results when using pituitary extract compared to synthetic preparations in African catfish reproduction.

In the present study, it was found that all indicators were significantly better in carp treated with pituitary compared to those treated with Nerestin-6.

A larger amount of row and a larger number of hatched prelarvae were obtained from them, the differences being statistically significant. The difference in absolute fecun-

Table 5. Reproductive characteristics in female carp from the different experimental groups

Indices	Neristin-6		Pituitary	
	Mean	Std. Dev.	Mean	Std. Dev.
Time of maturation	12.00	0.00	12.00	0.00
Absolute fecundity, eggs	583966 ^{b}	82433	961751 ^{b}	166325
Relative fecundity, eggs/g BW	140.6 ^{c}	5.877	152.1 ^{c}	6.978
Relative fecundity, eggs/L cm	952.7 ^{c}	100.8	1370.1 ^{c}	187.6
Egg fertility rates, %	73.23 ^{b}	4.507	92.50 ^{b}	1.997
Hatched prelarvae, %	32.50 ^{c}	4.275	54.50 ^{c}	2.330

^{b} $P < 0.01$, ^{c} $P < 0.05$

dity was 1.6 times higher (P <0.01) in favour of the pituitary-treated group; in relative fertility rate calculated to BW – 7.6% (P < 0.05), in relative fecundity calculated to fish length – 1.4 times (P < 0.05) and in hatched prelarvae – over 1.6 times higher (P < 0.05).

Carp belongs to the species of high fecundity. Different indicators are calculated for establishing fecundity of fish – absolute and relative fecundities (the number of vitellogenic oocytes per weight unit or body length), (Rizzo and Bazzoli, 2020). In carp, fecundity may vary considerably from one breed or population to other. Thus, in Ropshin carp, a relative fecundity of 110 – 130 thousand eggs per 1 kg of body weight was found (Miroshnikova & Ponomarev, 2013). In Uzbekistan carp, the absolute fecundity varied from 131 to 1830 thousand eggs and the relative fecundity from 190.5 to 344.0 eggs per 1 g of gutted fish BW (Kamilov et al., 2020).

In the present study, the highest absolute fecundity of common carp was 961,751 eggs and the relative – 152.1 eggs/kg BW.

Fecundity in the pituitary-treated group was very good (92.50%) and the difference to the group treated with Nerestin was 20.8%.

The number of larvae obtained in the group injected with pituitary suspension was 54.5%, while in the group injected with the universal preparation Nerestin-6 the result was significantly lower (32.5%).

The reason for the lower levels after the application of Nerestin-6 in carp may be related to the fact that the product is universal and it is recommended for different fish species.

The mechanisms of action of the pituitary suspension and the synthetic preparations are different. The pituitary is a source of gonadotropic hormones that directly affect the gonads, while Nerestin preparations are hypophysiotropic, they do not contain hormones or hormone-like substances and their effect is related to stimulating the gonadotropic systems of fish.

Based on that and taking into consideration the many benefits of Nerestin over the pituitary, research work on common carp should continue under the concrete conditions in order to study the effect of frequent, fractional injection schemes with Nerestin-6 for achieving optimal results.

Controversial results were reported in studies of various natural and synthetic preparations for carp reproduction. Thus, Brzuska (2006), comparing the effect of carp pituitary and two synthetic preparations – Ovopel and Dagin, on the propagation results in female individuals of carp, Hungarian strain 7, found statistically significant differences in fertility, the best results being obtained with Ovopel (94.87%), followed by carp pituitary (86.17%) and Dagin (80.11%). In the same study, the method of hormonal treatment did not have a significant effect on the percentage of living embryos, the results being, as follows: carp pituitary (74.44%), Ovopel (75.54%) and Dagin (71.83%).

In the study of Farag et al. (2017) on carp treated with pituitary, all the indicators characterizing fecundity were better compared to fish treated with Human Chorionic Gonadotrophin, Receptal and Ovaprim.

In a comparative study of treatment with pituitary and various Nerestin preparations in channel catfish (*Ictalurus punctatus*), Yushkova (2009) found better results after treatment with pituitary in some cases, while in other the results were in favour of Nerestin.

With regard to female herbivorous fishes, it can be seen that all the fishes from the experimental groups were well-developed and with optimal body weight (Table 4). The average body weight and the coefficient of condition in grass carp varied within 6513.0 to 7212.0 g and from 1.9 to 2.0, respectively, and in silver carp from 5949.0 to 6408.0 g and from 1.7 to 2.0, respectively. Development and reproductive characteristics of herbivorous fishes were the subject of research in different regions where they had been introduced. In a study of female silver carp in Uzbekistan, Kamilov & Komrakova (1999) found that fishes matured when they reached a body weight of 1.5 – 3 kg and a length of 42 – 55 cm. Silver carp in the Syrdarya River in Uzbekistan reached sexual maturity at a body weight of 4000 g and a body length of 60 cm in male fishes and 5000 g and 65 cm in the females, respectively (Kamilov et al., 1994).

Table 6 presents the results obtained in female grass carp, treated with various preparations. The time of maturation was from 12.06 to 12.38 h and there was no significant difference between the groups according to that indicator. It should be noted that according to all the studied indicators, the highest values were registered in the group injected with Nerestin-1, the lowest values in fish treated with Nerestin-6 and the group injected with the pituitary solution occupied an intermediate position.

The differences between the groups in relative fecundity calculated per kg of body weight were insignificant, in contrast to the relative fecundity calculated according to fish length. Here again the highest and the lowest values were found in fish injected with Nerestin. Those injected with Nerestin-1 showed 14.6% higher fertility, the difference being statistically significant.

The difference in EFR and HP between the fishes treated with Nerestin-1 and Nerestin-6 was 20 and 9.8%, respectively (P < 0.001) in favour of the former. The difference in those indicators between the group treated with pituitary extract and Nerestin-6 was 17.5 and 7.76%, respectively, in favour of the former.

Table 6. Reproductive parameters in female grass carp from different experimental groups

Characteristics	Nerestin-1		Nerestin-6		Pituitary	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
Time of maturation	12.06	0.537	12.38	0.098	12.27	0.404
Absolute fecundity, eggs	952108	69396	764234	73918	876413	198764
Relative fecundity, eggs/g BW	138.2	28.59	118.5	15.55	120.8	7.622
Relative fecundity, eggs/L cm	1157 [c]	68.59	988.2 [c]	93.12	1104	240.2
Egg fertility rates, %	92.57 [a]	1.150	72.57 [a,a1]	3.951	90.07 [a1]	2.479
Hatched prelarvae, %	53.03 [a]	1.704	43.27 [a,a1]	2.686	51.03 [a1]	3.139

[a, a1] $P < 0.001$, [b] $P < 0.01$, [c] $P < 0.05$

In a comparative study of Nerestin-6A and carp pituitary in *Perca fluviatilis*, Barbacariu et al. (2018) found significantly higher amounts of harvested eggs in Nerestin-6A-treated fishes compared to carp pituitary-treated fishes.

In a study of the effect of the synthetic preparation Ovaprim-C on the reproductive performance in grass carp, Naeem et al. (2011) found an advantage of Ovaprim over pituitary extract. The relative fecundity established in the study was 62532 eggs/kg BW; egg fertility rate 80.36% and hatchability 79.49%.

In the present study the relative fecundity was significantly higher in all the three experimental groups and the egg fertility rate was higher in the group treated with Nerestin-1 and pituitary extract. Hatchability in the experiment was significantly lower than that reported by Naeem et al. (2011).

In a comparative study of the application of LHRH-a and pituitary extract in induced breeding of grass carp, Jha & Neupane (2019) found that the egg fertility and hatchability were better when using the synthetic preparation. In that study, the relative fecundity per kg BW in grass carp was 125000 eggs, fertility rate 40 – 70% and the hatched prelarvae from fertilized eggs 60 – 70%. In terms of relative fecundity, the results were close to those obtained in the present study and in terms of egg fertility and hatchability the results were lower than those obtained by us.

Table 7 presents the results related to the reproductive characteristics of silver carp in the different experimental groups. As with carp, there was no difference between the groups concerning maturation. All the fishes matured in 12 hours.

The lowest AF, RFg and RFcm were reported in fish treated with Nerestin-6 and the highest – in those treated with pituitary extract but the differences between the groups were insignificant. Significant differences between the groups were found for EFR and HP. The highest egg fertility rate and hatchability was obtained in fish treated with Nerestin-1 and the lowest in those treated with Nerestin-6, the difference being 14.4% ($P < 0.05$) and 17.9% ($P < 0.05$), respectively. The difference between the fish treated with Nerestin-6 and those treated with the pituitary extract was 9.8% ($P < 0.05$) and 14.4% ($P < 0.05$), respectively, in favour of the latter.

Egg fertility rates of fish from the same species can vary considerably. Kamilov & Komrakova (1999) reported an absolute fecundity of 0.5 – 1.9 million eggs in silver carp and a relative fecundity within the range of 95 – 458 eggs per g of gutted fish. In another study of silver capr from the the Syrdarya River in Uzbekistan, Kamilov et al. (1994) found an absolute fecundity in the range of 1260 to 5400 thousand eggs and a relative fecundity of 79 to 392.3 eggs per g of gutted fish weight. Comparing the above results about silver carp fecundity with those obtained in the present study, it becomes clear that the characteristics can vary significantly in fishes from different populations.

Table 8 presents the results of the comparison of the studied characteristics between the different species within each variant of hormonal treatment.

Table 7. Reproductive characteristics in female silver carp from the different experimental groups

Characteristics	Nerestin-1		Nerestin-6		Pituitary	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
Time of maturation	12.00	0.000	12.00	0.000	12.00	0.00
Absolute fecundity, eggs	821542	110786	754121	77822	852378	74890
Relative fecundity, eggs/g BW	130.6	16.14	126.47	6.364	143.8	11.96
Relative fecundity, eggs/L cm	1063	62.06	953.6	77.24	1085	82.48
Egg fertility rates, %	88.00 [c]	2.773	75.30 [c, c1]	3.345	83.44 [c1]	2.573
Hatched prelarvae, %	50.20 [c]	1.931	41.23 [c, c1]	2.721	48.14 [c1]	1.352

[a] $P < 0.001$, [b] $P < 0.01$, [c; c1] $P < 0.05$

Table 8. Differences by characteristics when comparing individual fish species within each variant of hormonal treatment

Preparation	Comparison between species*	Characteristics											
		Time of maturation		AF, eggs		RFcm, eggs		RFcm, eggs		EFR, %		HP, %	
		Difference between species											
		Mean Diff.	Sig.	Mean Diff.	Sig.	Mean Diff.	Sig.	Mean Diff.	Sig.	Mean Diff.	Sig.	Mean Diff.	Sig.
Pituitary	Sc - Gc	-0.267	0.211	-24059	0.856	+22.91 [c]	0.022	-18.32	0.906	-6.631 [c]	0.014	-2.899	0.188
	Cc - Gc	-0.267	0.211	+85338	0.527	+31.220 [b]	0.006	+266.5	0.124	+2.433	0.254	+3.467	0.126
	Cc - Sc	0.000	1.000	+109397	0.423	+8.309	0.308	+284.7	0.104	+9.064 [b]	0.003	+6.365 [c]	0.017
Nerestin-6	Sc - Gc	-0.387 [a]	0.000	+10113	0.879	+8.021	0.348	-34.63	0.660	+2.733	0.435	-2.033	0.496
	Cc - Gc	-0.387 [a]	0.000	-180268 [c]	0.020	+22.16 [b]	0.021	-35.54	0.630	+0.658	0.837	-10.767 [b]	0.005
	Cc - Sc	0.000	1.000	-170155 [c]	0.025	+14.138	0.100	-0.908	0.990	-2.075	0.523	-8.733 [c]	0.013
Nerestin-1B	Sc - Gc	-0.057	0.864	-130566	0.159	-7.557	0.710	-94.90	0.150	-4.567 [c]	0.058	-2.833	0.129

[a] P < 0.001, [b] P < 0.01, [c] P < 0.05; *Cc – Common carp; Gc – Grass carp; Sc – Silver carp

When applying pituitary extract and Nerestin-1, no significant differences in the time of maturation of the fishes were found. When Nerestin-6 was used, there was no difference in the performance between common carp and silver carp, but maturation was significantly delayed in grass carp (P <0.001) compared to the other two species.

Significant differences were not found between the species according to RFcm in the studied variants of hormonal treatment.

After treatment with pituitary extract, the highest fecundity, egg fertility rate and hatchability were reported in common carp. It showed better absolute and relative fecundity, the difference in RFg compared to grass carp being statistically significant (P < 0.01). Within the variant, silver carp had higher RFg compared to grass carp (P < 0.05). Referring to EFR, grass carp and common carp showed significantly better characteristics than silver carp (P < 0.05). A statistically significant difference between silver carp and common carp was also established referring HP, in favour of the latter (P < 0.05).

After treatment with Nerestin-6, the results differed compared to treatment with pituitary. Within the variant, the highest AF was reported for grass carp and the lowest for common carp. Common carp had significantly lower AF (P < 0.05) compared to both herbivorous fish species. Referring to RFg, on the contrary, common carp had the highest values within the variant, the difference to grass carp being significant (P < 0.05). The best EFR results within the variant were reported for silver carp and the lowest for grass carp, but the differences between all the studied species were insignificant.

In contrast to EFR, the highest HP values within the variant with Nerestin-6 application were established in grass carp and the least hatched prelarvae were reported in common carp.

Within the variant, common carp showed significantly lower hatchability performance both compared to grass carp (P < 0.01) and to silver carp (P < 0.05).

In the variant with Nerestin-1, used in herbivorous species, a significant difference (P < 0.05) was reported in egg fertility rates in favour of grass carp.

Conclusions

A comparative study of the reproductive indicators after treatment with pituitary extract and Nerestin in artificial spawning of common carp, silver carp and grass carp from local populations in Kazakhstan showed a certain specific characteristics in the different species.

Pituitary-treated common carp fishes had better reproductive parameters compared to those treated with Nerestin-6, the differences being statistically significant. Significantly larger amount of eggs and a larger number of hatched prelarvae were obtained.

In grass carp, the highest values were reported in the group injected with Nerestin-1, the lowest – in fishes treated with Nerestin-6 and the group injected with the pituitary extract took an intermediate position. The differences in relative fecundity between the two groups treated with Nerestin, calculated to BL by EFR and HP were 14.6% (P < 0.05), 20 and 9.8% (P < 0.001), respectively. The difference in EFR and HP between the groups treated with pituitary extract and Nerestin-6 was 17.5 and 7.76%, respectively (P < 0.001) in favour of the former.

In silver carp, the lowest AF, RFg and RFcm were reported in fish treated with Nerestin-6 and the highest in those treated with pituitary extract, but the differences between the groups were insignificant. Significant differences between the groups were found for EFR and HP. The highest egg fertility rate and hatchability was obtained in fish treated with Nerestin-1 and the lowest in those treated with Nerestin-6, the difference being 14.4% ($P < 0.05$) and 17.9% ($P < 0.05$), respectively. The difference between the fishes treated with Nerestin-6 and those treated with the pituitary extract was 9.8% ($P < 0.05$) and 14.4% ($P < 0.05$), respectively, in favour of the latter.

The comparative analysis of the separate species within each variant showed that the highest fecundity, egg fertility rate and egg hatchability were observed in common carp after treatment with pituitary extract. No significant differences in EFR were observed between the studied species after treatment with Nerestin-6. Within each variant, common carp showed higher RFg compared to grass carp and silver carp, but the lowest hatchability, which was statistically significant. When Nerestin-1 was used in herbivorous fishes, the best results were obtained in grass carp, the difference in EFR with silver carp being significant ($P<0.05$). Summarizing the results obtained, it may be recommended to apply pituitary extract for inducing artificial spawning in common carp and Nerestin-1 in grass carp and silver carp. Taking into consideration the many advantages of Nerestin over the pituitary extract, it is advisable to continue research with common carp under the concrete conditions and to test schemes with frequent, fractional injection of Nerestin-6 for achieving optimal results.

Acknowledgments

The present study was supported by the Ministry of Ecology, Geology and Natural Resources of the Republic of Kazakhstan (Project No. BR10264236).

References

Acuña J.J.A. & Rangel, J.L.H. (2009). effects of hypophysial extract of Common carp and the analog of the GNRH on the final maturation oocyte and the spawning of Cachama negra (*Colossoma macropomum). Revista Cientifica de Veterinaria*, *19(5)*, 486-494.

Alpejsov, Sh.A., Fedorov E.V., Kojshabaeva, S.K. & Badryzlova, N.S. (2015). Prospects for the development of pond fish farming in Kazakhstan. *Research, Results*, *3*, 19-23, (Ru).

Anonymous (2021). On the development of fishery. Resolution of the Government of the Republic of Kazakhstan dated April 5, 2021. № 208. *https://adilet.zan.kz/rus/docs/P2100000208,* (Ru).

Anonymous (1986). Collection of regulatory and technological documentation on commodity fish farming. Moscow, Agropromisdat, *1*, 1261, (Ru).

Assylbekova, S.Zh. & Kulikov, E.V. (2016). Introduction of fish and aquatic invertebrates in reservoir of Kazakhstan: results and perspectives. *Vestnik AGTU, Fish farming*, *3*, 16-29, (Ru).

Assylbekova, S.Zh., Isbekov, K.B. & Kulikov, E.V. (2014). Guidelines for environmental users and farmers on LCFE (Lakes - commercial fisheries enterprise). *Methodical manual, Almaty, Kazakhstan,* 129, (Ru).

Assylbekova, S.Zh., Isbekov, K.B., Kulikov, E.V. & Nevalennaj, A.N. (2018). Acclimatization of fish and aquatic invertebrates in the water bodies of Kazakhstan. *Monograph, Almaty, Kazakhstan,* 239, (Ru).

Assylbekova, S.Zh., Kulikov, E.V., Kulikova, E.B. & Tsoi, V.H. (2013). Metodical guidelines for environmental users and farmers on LCFE (Lakes – commercial fisheries enterprise), 171, *Methodical manual, Almaty, Kazakhstan,* (Ru).

Barbacariu, C.A., Dirvariu, L., Burducea, M. & Mihai, C. (2018). Comparative study on the use of various hormonal preparations (carp pituitary, Nerestin 6A) in the artificial reproduction of *Perca fluviatilis*. *Scientific Papers - Animal Science Series*, *70*, 161-166, *70*. https://www.uaiasi.ro/firaa/Pdf/Pdf_Vol_70/CA_Barbacariu.pdf

Brzuska, E. (2006). Artificial propagation of female Hungarian strain 7 carp (*Cyprinus carpio*) after treatment with carp pituitary homogenate, Ovopel or Dagin. *Czech Journal Animal Science*, *51(3)*, 132–141.

Dima, R.C., Patriche, N., Talpeş, M., Tenciu, M. & Dicu, D.M. (2009). Comparative study on the hormonal stimulation Sterlet (*Acipenser ruthenus*, Linnaeus, 1758) using carp hypophysis and artificial hormone type Nerestin 5-5A. *Scientific Papers Animal Science and Biotechnologies, 42(2*), 28-32,(Eng).

FAO (2020). The state of world fisheries and aquaculture 2020. Sustainability in action. Rome, 206, https://doi.org/10.4060/ca9229en

Farag, M.E., Zeinhom, M.M. & Ibrahim, I.H. (2017). Stimulation spawning of Common carp, Grass carp and Silver carp by carp pituitary extract, human chorionic gonadotrophin, receptal and ovaprim hormones for commercial purposes. *Proceedings of „1st International Conference (Central Laboratory For Aquaculture Research In Cooperation With Worldfish“*, Cairo, Egypt, 20-22 November, 2017, *2*, 325-346.

Grozev, G, Hadjinikolova, L., Boyadzhiev, A. & Petrov, P. (1999): Freshwater fish farming, Demi Publishing House, Plovdiv, 268, (Bg).

Gvozdev, E.V. (1988). Fishes of Kazakhstan. Nauka: Almaty, Kazakhstan, *3*, 304, (Ru).

Jha, B.S. & Neupane, S. (2019). Induced breeding of Grass carp, *Ctenopharyngodon idella* (Valenciennes) in Rupandehi, Nepal. *ZOO-Journal*, *5*, 82–84.

Kamilov B., Salihov, T. & Madjid, **Z.** (1994). Reproductive capacity of silver carp, *Hypophthalmichthys molitrix* (Val.) from the River Syrdarya in Uzbekistan. *Turkish Journal of Zoology*, *18(2)*, 111-114.

Kamilov, B.G. & Komrakova, M.Y. (1999). Maturation and fecundity of the silver carp, *Hypophthalmichthys molitrix*, in Uzbekistan. *The Israel Journal of Aquaculture - Bamidgeh*, *1*, 40-43.

Kamilov, B.G., Yuldashov, M.A., Soatov, U.R. & Toshova, N. (2020). Common carp maturation and fecundity and reproduction optimization in Uzbekistan. *IOP Conference Series: Earth and Environmental Science*, *614(1),* 012155. doi:10.1088/1755-1315/614/1/012155

Kozlov, V.I. (1998). Fishery Farmer's Handbook. VNIRO, 342,(Ru).

Kulikov, Y.V. & Assylbekova, S.Zh. (2020). The fisheries and aquaculture sector in Kazakhstan.EUROFISH-Magazine, *3*, 53-55. http://eurofishmagazine.com/magazine-issues/current-issue/item/728-eurofish-magazine-issue-3-2020-may-june

Lyubomirova, V.N., Shlenkina, T.M., Rakova, L. Yu. & Fatchutdinova, Yu.V. (2020). Comparative appraisal of hormonal inductors of artificial spawning of female of African Sharptooth Catfish. *Vestnik UGSA*, 71-78.

Miroshnikova, E.P. & Ponomarev, S.V. (2013). Aquaculture: Practicum, OGU, Orenburg, 184, (Ru).

Naeem, M., Zuberi, A., Salam, A., Ashraf, M., Elahi, N., Ali, M., Ishtiaq, A., Malik, T., Jamshed Khan, M., Mazhar Ayaz, M., Javed Iqbal, M. & Ahmad, B. (2011). Induced spawning, fecundity, fertilization rate and hatching rate of Grass carp (*Ctenopharyngodon idella*) by using a single intramuscular injection of ovaprim–C at a fish hatchery Faisalabad, Pakistan. *African Journal of Biotechnology*, *10(53),* 11048-11053. doi: 10.5897/ajb10.1481

Popescu, A., Crețu, M., Nica, A. & Ibănescu, D.C. (2018). Induced breeding of *H. molitrix* and histological observations on the development stage of oocyte. *Journal of Agroalimentary Processes and Technologies*, *24(4)*, 325-329.

Radu, D., Costache, Mih., Costache, M., Marica, N. & Dobrota, N. (2018). Research on reproductive performance of carp breeds (*Cyprinus carpio* L.) Frasinet, Ineu and Ropsa. In: *Proceedings of the „18th International Multidisciplinary Scientific GeoConference", SGEM* 2018, 02-08 July, 2018, *18(6.2)*, 513-520. doi:10.5593/sgem2018/6.2/s25.068

Rizzo, El. & Bazzoli, N. (2020). Reproduction and embryogenesis. In: *Biology and Physiology of Freshwater Neotropical Fish*, Ch. *13*, 287-313.

Ryzhkov, L.P. (1987). Lake fish farming. Agropromizdat, 336, (Ru).

Weber, G.M. (2011). Use of technologies to control reproduction in finfish aquaculture and feed the world. *Biology of Reproduction*, *85(1)*, 108, https://doi.org/10.1093/biolreprod/85.s1.108

Weber, G.M. & Cheng-Sheng, **Lee** (2014). Current and future assisted reproductive technologies for fish species. *Advances in Experimental Medicine and Biology*, *752*, 33-76. doi: 10.1007/978-1-4614-8887-3_3

Woynarovich, A., Moth-Poulsen, T. & Péteri, A. (2010). Carp polyculture in Central and Eastern Europe, the Caucasus and Central Asia: a manual. *FAO Fisheries and Aquaculture Technical Paper*, Rome, FAO, *554*, 73.

Yushkova, Y.A. (2009). Biotechnics of reproduction and rearing of juvenile clarias catfish in polycycle mode in a closed water supply installation. PhD Thesis, Orel State Agricultural University, 127, (Ru).

Zalepuhin, V.V. (2009). Optimization of quality assessment of carp fish breeders in aquaculture. *Dr. Sci. Biol. Thesis*, Russian Federal Research Institute of Freshwater Fisheries (VNIIPRH), 527, (Ru).

Zohar, Y. & Mylonas, C.C. (2001). Endocrine manipulations of spawning in cultured fish: from hormones to genes. *Aquaculture*, *197*, 99–136.