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Biotechnology of hormone stimulation of the maturation of  
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by A. A. Boev, R. N. Stepanova, and  
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Biotechnology of Hormone Stimulation of the Maturation of Bream in Relation to Intensified Bream Rearing in the Volga Delta.

By

A. A. Boev, R. N. Stepanova and B. G. Travkin.

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During recent decades the hydrological conditions in many of our country's water bodies, which are important to the fish industry, have considerably changed. This has had a particularly adverse effect upon the efficiency of the natural spawning of many species of commercial fish. Therefore, in recent years greater and greater attention has been given to the artificial reproduction of commercial fish on an industrial (factory) basis. The reproduction of semi-diadromous fish (carp, bream and zander) is mainly carried out in accordance with the earlier extensive pattern of the fish breeding process. The growing requirements of the national economy demand continuous intensification of fish rearing; one of the most important ways to increase the efficiency of spawning nursery farms is to convert them to factory or semi-factory methods of operation, in which mature sex cells are obtained by means of the method of hypophyseal injections. The

conversion of spawning nursery farms to a fundamentally new pattern of pisciculture is possible only after a thorough development and verification of basic biotechnological norms under production conditions for each water body and individual fish species.

Research has now been completed on the main aspects of the biotechnology of the factory reproduction of semi-diadromous fish, carp and zander, in various water bodies in our country (Fiser, 1955; Voynarovich, 1959; Konradt, Sakharov, 1963; Boyko, Makarov, 1963; Konradt, Sakharov, 1969; Barannikova, Boev, Garlov, Saenko, 1969; Boev, Travkin, 1969; Landyshevskaya, 1972; Popova, Frolova, 1972).

The possibility of obtaining bream larvae by means of the method of hypophyseal injections, and the raising of juveniles in ponds, were demonstrated in 1969-1970 in the Volga Delta (Boev, Stepanova, Travkin, 1971; Stepanova, 1972; Boev, Stepanova, Travkin, 1973). On the basis of these studies it was shown that the biotechnology of obtaining bream larvae by the factory method consists of the following stages: procurement of brood stock; holding of the brood stock until the moment of injection; injection of the brood stock; production, fertilization and removal of mucilage from eggs; incubation of eggs and hatching of larvae; keeping of larvae until their transition to exogenous feeding; transplanting larvae into ponds and raising juveniles.

Procuring bream brood stock in the lower reaches of the Volga River presents no difficulties. Within a short period of time, from 10th to the 30th of April practically any amount of bream may be procured for pisciculture purposes. Our operational experience has shown that the largest bream brood stock have high working fecundity (on the average 120 to 150 thousand eggs) and mature excellently from hypophyseal injections. M. A. Letichevskiy (1952) points out that the working fecundity of bream females

increases with the length of the females. V. A. Panasenکو (1972) has demonstrated in the Courland Lagoon that a direct relation exists between the viability of bream eggs and the age of bream brood stock; the highest egg survival rate has been recorded in 8- to 10-year-old brood stock:

Bream brood stock must be procured during its main run into the Volga within a short period of time; the time the brood stock is held in live fish carrier (boats) until it is transplanted to ponds must be cut to a minimum. Male and female bream up to the moment of injection are kept separately in small pre-spawning ponds with an area of 0.2 to 0.5 hectares, with a maximum circulation (through-flow) rate and a water temperature somewhat below the spawning temperature. Under such holding conditions, even after 15 days in the ponds, female bream mature well from hypophyseal injections and the obtained eggs have a high percentage of fertilization.

Injection of the brood stock began when the water temperature in the tanks for holding injected brood stock went no lower than 16°C or even in the early morning hours. If, 24 hours prior to injection, the female bream, are kept at spawning temperature (16 - 18°C), then the number of females maturing from hypophyseal injections will be approximately 100%, and the quantity of fertilized eggs will, as a rule, be not less than 90%. Operations with bream during two seasons have shown that not only should the dose of a preparation of acetonized hypophyses be calculated on the basis of its [presumably the preparation's] weight units, but that strict allowance should also be made for the gonadotropic activity of the hypophysis preparation expressed in biological units (in this case in frog units). One and the same amount by weight of a preparation of acetonized hypophyses may contain a different quantity of a hormone stimulating maturation and ovulation. Therefore, if the biological activity of a preparation of hypophyses is disregarded when injecting brood stock, it is easy to err either by giving

an overdose, which may lead to a decrease in egg quality, or by giving an insufficient dose, which will result in a sharp decline in the percentage of mature females, even though the amount by weight of preparation may correspond exactly to that recommended. When injecting bream brood stock we used a tested preparation of acetonized hypophyses of carp<sup>\*)</sup>. The mass of female bream fluctuated within the range of 900 to 1,200 grams. The optimal doses of preparation were found according to the following schedule (Table 1).

During the maturation of the females the water temperature fluctuated within a range of 16 to 18°C. The obtained results permit us to conclude that 10 to 12 frog units per kilograms of a female's mass represent optimal doses of a preparation of acetonized hypophyses of carp at the above strength. When all the foregoing recommendations are fulfilled the maturation of bream females from hypophyseal injections is almost 100%, and the amount of

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Table 1

Experiment on the injection of bream brood stock with a tested preparation of acetonized hypophyses of carp.

Таблица 1

Опыт по инъекированию производителей леща тестированным препаратом ацетонированных гипофизов сазана

1 № варианта опыта	2 Доза препарата гипофизов на одну самку. Л. Е.	3 Число сам- ок леща в варианте опыта	4 Число со- зревших самок	1 № варианта опыта	2 Доза препарата гипофизов на одну самку. Л. Е.	3 Число сам- ок леща в вариан- те опыта	4 Число со- зревших самок
1	2	10	0	3	8	10	8
2	4	10	0	4	12	10	10

Key to Table:

- 1 - No. of experiment variant;
- 2 - Dose of hypophysis preparation per female, in frog units;
- 3 - No. of bream females in experiment variant;
- 4 - No. of mature females.

<sup>\*)</sup> The testing was carried out on common male frogs (Rana temporaria); the biological strength of the preparation was 2 frogs units per one milligram of preparation.

fertilized eggs, as a rule, exceeds 90%. If the water temperature is raised to 18-24°C at the moment when the brood stock is injected the female bream mature well as doses of 8 frog units per 1 kilogram of fish mass, and the maturation time is decreased to 18-16 hours. The males mature well from doses of 4 frog units at water temperatures of 16 - 18°C; at higher temperatures they mature from doses of 2 frog units. By holding male bream at a temperature of 18 - 24°C for a period of 3 to 5 days, they enter maturation stage V without being subjected to hormonal treatment. The female/male bream ratio in the factory method of producing larvae may be 2:1. After hypophyseal injections bream mature together within similar lengths of time. [In our experiments the eggs from six to eight females were strained into a single basin and inseminated by the "dry" method with the sperm of three or four males; their mucilage was then removed by solutions of hyaluronidase and then tannin. Depending on the temperature of such solutions and process of mucilage removal lasts a total of 40-60 minutes and is the most labourious process when working with both carp and bream.

The viability of demucilaged eggs, and, consequently, the efficiency of the factory method of operating with bream and carp, depend to a considerable degree upon the skill of the fish culturist in this process. Until the process of mucilage removal from eggs is mechanized, the factory method of reproducing ordinary (run-of-the-mill) fish will involve considerable fluctuations in the survival rate of demucilaged eggs, and above all will remain very labourious. For incubating demucilaged bream eggs the Weiss apparatus with 8-10 litre capacity is, we believe the most suitable. With a batch of 2.5 - 3 litres of bream eggs in the Weiss apparatus water consumption of 3 - 3.5 litres per minute, and a water temperature of 16 - 20°C the incubation of the eggs progresses successfully. Larvae yield is 90% and

up. O. N. Vasil'chenko (1970) points out that in Volga Delta bream only individual oocytes are resorbed prior to spawning, while in carp the percentage of oocytes in a state of degeneration before spawning may be as high as 5%.

At a water temperature of 16-18°C bream eggs incubate in about 164 hours. Larvae hatched in Weiss apparatuses do not manifest any great tendency to settle on the bottom or to attach themselves to the walls of the apparatus (as do carp larvae), but they form columns [literally "candles"], are carried out of apparatus by the water current and accumulate in larva traps. As the larvae concentrate, they are scooped out and placed in holding nets of capron [nylon] mesh installed in concrete basins with slow water flow. Here the larvae are kept three to four days, and when they go over to exogenous feeding they are released into ponds to grow.

We raised larvae and juveniles for one month in ponds with an area of 0.04 to 0.6 hectare. To control larva quality, throughout the entire growing period we observed the state of the pond food supply, the way the fish fed, and the growth rate of the larvae and juveniles. Growth results are shown in Table 2.

Data obtained from the raising operation indicate a high survival rate and normal development of juvenile bream: the larvae and juveniles feed actively, grow well and are morphologically well formed. The yield of four-day old larvae over a 30-33 day period of cultivation in the ponds was 81 - 92%, and the maximum average mass was 0.24 gram.

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These data show that bream larvae and juveniles can actually be produced in the Volga Delta. This will permit us to improve the biotechnological process in spawning/nursery farms by gradually converting them to operation on the lines of the industrial fish hatchery.

Table 2

Experiments in raising larvae and juveniles in ponds with an area of 0.04 to 0.6 hectare

Таблица 2

Опыты по выращиванию личинок и молоди в прудах площадью 0,04—0,6 га

1. № пруда	2. Плотность посадки	3. Возраст, сут	4. Выход, %	5. Средняя масса, мг	6. Средняя длина, мм	7. Рыбопродуктивность, кг/га
1	8000 (200 т/га)	34	87,5	197,0 ± 4,2	28,2 ± 0,15	34
2	10 000 (250 т/га)	37	81,0	239,0 ± 4,1	30,3 ± 0,22	48
3	20 000 (500 т/га)	37	92,0	72,3 ± 5,0	21,1 ± 0,47	32
4	8000 (200 т/га)	34	87,7	204,0 ± 6,0	29,0 ± 0,21	35

Key to Table:

- 1 - No. of pond;
- 2 - Stocking density;
- 3 - Age, in days;
- 4 - Yield, in %;
- 5 - Average mass, in milligrams;
- 6 - Average length- in millimetres;
- 7 - Yield in kilograms of fish per hectare;
- 8 - Tonnes per hectare.

C O N C L U S I O N

The high efficiency, in all areas of biotechnology, of the factory method of raising bream in the Volga Delta will permit us to decrease by many times the number of required brood stock as compared to the enormous number now used for natural spawning at spawning/nursery farms in order to produce the millions of bream fingerlings required by government plans.

Planting of three- and four-day-old viable bream larvae in the bayous of fish farms, and the resultant absence of contact between the larvae and brood stock, will decrease the possibility of epizootics and will prevent the developing juveniles from being devoured by the brood stock and from competing with the latter for food. All these positive factors must contribute to a major increase in the fish yield from the flooded areas of fish farms.

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## Summary:

Biotechnik of hormonal stimulation of *Abramis brama* maturation in connection with the intensification of its rearing in the Volga gulf

Boev A. A., Stepanova R. N., Travkin B. G.

## SUMMARY

The principal questions of receiving of *Abramis brama* larvae at the factory by the method of hypophysial injections in the Volga's delta are considered. The results of larvae growth and fingerlings in ponds in the Astrakhan region (after using of hypophysial injections to adult fishes) shows the possibility of the biotechnic of *Abramis brama* rearing and reproduction in Volga's delta.