

Effect of different commercial spawning agents on the effectiveness of pike-perch, *Sander lucioperca* (L.), reproduction under controlled conditions in Ukraine

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Abstract. The technology of pike-perch (*Sander lucioperca*) aquaculture faces problems with its reproduction, which is the unsynchronized ovulation, even among simultaneously injected females, and involuntary spawning. Synchronization of spawning can be achieved by using new, modern hormonal drugs to stimulate oogenesis. The aim of this study was to determine the effect of the drugs "Surfagon", "Vadilen", and pituitary hormone injections on the reproductive system of *S. lucioperca* kept in aquaculture. Brood fish were kept in a recirculation aquaculture system and evaluated according to parameters as: body weight (kg), body length (cm), body height (cm), body girth (cm), average fatness index. The effect of drugs on females was evaluated by the following indicators: produced egg weight (g), number of eggs in 1 g, working fertility (thousands eggs), eggs obtained (in % of body weight). The effect of drugs on males was evaluated by the following indicators: volume of ejaculate (mL), duration of active translational movement of spermatozoa (s), working fertility (million specimens of spermatozoa) and the number of live spermatozoa, from the total number (%). The best results for the studied *S. lucioperca* specimens were provided by using commercial spawning agent "Vadilen", which obtained an egg weight of 7.8% of the female weight. The drug "Surfagon" was the second most effective, which obtained an egg weight of 7.4% of the female weight. The lowest results, an egg weight of 6.9% of the female weight, were recorded with the pituitary preparation treatment.

Key Words: brood fish, ejaculate, fertilization, larval survival "Surfagon", "Vadilen", pituitary hormone.

Introduction. Pike-perch (*Sander lucioperca*) is a freshwater fish from the perch family (Percidae), typical for predatory species of the ichthyofauna of lakes and rivers. This species, which is able to withstand low water salinity, can be found in the northern seas and in the Black Sea, which is of interest for the aquaculture of Ukraine. In general, pike-perch is a native and fairly widespread species in the reservoirs of Western Europe, and due to acclimatization works, its range of existence has expanded significantly. Today it is found in Algeria, Morocco, Tunisia, China and Kyrgyzstan, although the degree of success of its cultivation varies significantly between countries (Reshetnikov 1998; Craig 2000; Rottmann et al 1991; Welcomme 1988).

In modern ichthyology, researchers distinguish at least five main related species of pikeperch, the biology of which is similar to each other, namely: common pike-perch or zander (*S. lucioperca*), yellow pike or walleye (*Sander vitreus*), Canadian pike-perch or sauger (*Sander canadensis*), sea pike-perch or estuarine perch (*Sander marinus*) and Volga pike-perch (*Sander volgensis*) (Kottelat & Freyhof 2007). For decades, pike-perch has traditionally been cultivated in ponds, in Ukrainian aquaculture. It was mostly used as an additional object in polyculture with carps (Horváth et al 1984; Salminen & Ruuhijärvi 1991; Verreth & Kleyn 1987; Hrynzhovskyi et al 2001; Tretiak et al 2008).

Under the influence of anthropogenic load (primarily overfishing, poaching, regulation and river pollution), the volume of *S. lucioperca* catch has been steadily decreasing recently, despite the rising market demand (FAO 2015; Kotovska 2011). The

research on this species focuses on growing methods in fish farming conditions, using semi-intensive or intensive technologies, in particular on how to increase the efficiency of *S. lucioperca* reproduction (Demska-Żakęś & Żakęś 2002; Kucharczyk et al 2007; Németh et al 2009; Rónyai 2007; Żakęś 2007). The least effective methods of working with *S. lucioperca* are environmental stimulation by increasing the temperature and/or changing the lighting regime (Müller-Belecke & Zienert 2008; Wang et al 2010). Slightly better results were provided by using preparations from the pituitary gland of carp and herbivorous fish species for hormonal stimulation of *S. lucioperca* brood. However, the use of the above-mentioned drugs initiates the emergence of two significant problems: asynchronous ovulation among simultaneously injected females and involuntary spawning inside the fish tank, which leads to a complete loss of reproductive material (Żarski et al 2012; Sarameh et al 2012; Steffens et al 1996; Lappalainen et al 2003; Rottmann et al 1991; Żakęś & Szczepkowski 2004; Schlumberger & Protea 1996).

Research on neurosecretion in the hypothalamus, carried out at the end of the 1930s, made it possible to solve these problems and became the basis for the study of neurohormonal regulation of gonadotropin secretion by the hypothalamic hormone, which was performed at the end of the 1960s (Goncharov 1977). As a result, the above-mentioned studies made it possible to synthesize drugs that have been used for more than 20 years to stimulate the maturation of fish sexual products in most countries of the world. The most promising among the above-mentioned hormonal drugs are synthetic analogues of the hypothalamus releasing hormone - gonadotropin-releasing hormone (GnRH), which is the highest link of the central axis of the regulation of the reproductive cycle in fish. The use of these drugs makes it possible to completely abandon injections of the drug into the pituitary gland of fish whose germ cells are in the final stages of gametogenesis (Goncharov et al 1991; Nagahama 1994; Das 2004; Kucharczyk et al 2005).

In the Ukrainian aquaculture conditions, the drug "Surfagon" and its conceptual successor - "Vadilen" were the most widely used. Ovulation and spermiation in fish under the influence of these drugs are achieved due to a mild effect on the pituitary gland in order to stimulate further secretion of its own gonadotropins. This way, better results are achieved than when using pituitary injections (Baltadzi et al 2001; Kovalenko & Kulish 2006; Shumova & Kovalenko 2009; Kovalenko 2003; Kovalenko 2008). Thus, in the conditions of Ukrainian fish farms, it has been proven that these drugs have a number of undeniable advantages, such as the stability of composition and action, absence of side effects, extended shelf life, significantly lower cost compared to natural drugs, stable availability on the market (Kovalenko et al 2013). However, there are almost no studies of their effect on pike-perch, because it is not a traditional species for the intensive aquaculture in Ukraine and whose cultivation is not carried out in ponds, but in pools or recirculation aquaculture systems. Thus, since *S. lucioperca* is a promising species for the aquaculture, with an increasing in demand on the market, the purpose of this study was to characterize the feasibility of using the drugs "Surfagon" and "Vadilen" during its spawning campaign.

Material and Method

The research was carried out on the basis of the Institute of Fisheries of Ukraine and PrJSC "Khmelnyskrybhosp". The experiment was carried out in the conditions of the incubation workshop of PrJSC "Khmelnyskrybhosp", located in the village of Medzhibyzh, Khmelnytskyi region of Ukraine. One control and two research groups were involved in the experiment. In each group, drugs were injected using a medical syringe, through the back muscle, above the lateral line. The scheme for injecting the *S. lucioperca* in their breeding groups is presented below, in Table 1.

In the control group, carp pituitary gland injections were used to stimulate the maturation of sexual products. Injections were prepared by means grinding the pituitary gland and then dissolving it in 0.5% saline solution (5 g of NaCl dissolved in 1 mL of distilled water). 1 mL of physiological solution was used for 4 mg of dry pituitary substance. 1 mL of the pituitary preparation was used per 1 kg of live fish weight (Kononenko et al 2016; Davydov et al 1992).

Table 1

Scheme of injections for *Sander lucioperca* breeders

Group of research	Drug dosage, mL		
	First 10%	Final 90%	Total 100%
Experimental group I, "Surfagon"		♀	
	0.3	2.95	3.25
	0.3	2.9	3.2
	0.2	2.1	2.3
	0.25	2.7	2.95
	0.2	2.2	2.4
		♂	
	1.7	1.7	3.4
	1.6	1.6	3.2
	1.85	1.85	3.7
Experimental group II, "Vadilen"	First 30%	Final 70%	Total 100%
		♀	
	0.18	0.42	0.6
	0.18	0.42	0.6
	0.15	0.35	0.5
	0.16	0.39	0.55
	0.18	0.42	0.6
		♂	
	0.54	0.54	1.08
	1.1	1.1	2.2
0.6	0.6	1.2	
Control, pituitary hormone	First 0%	Final 100%	Total 100%
		♀	
	—	7.5	7.5
	—	9.2	9.2
	—	4.9	4.9
	—	9.3	9.3
	—	10.2	10.2
	—	8.8	8.8
		♂	
	—	0.5	0.5
—	0.62	0.62	
—	0.54	0.54	
—	0.85	0.85	

In the experimental groups, injections of the drugs "Surfagon" and "Vadilen" were used to stimulate the maturation of sexual products. For the first time in Ukraine, in experimental group No. I, the drug "Surfagon" was used to stimulate the maturation of *S. lucioperca* eggs and milt according to a scheme similar to that for sturgeon and carp fish species (Hrynzhevskiy et al 2006). Injections were prepared by means of dissolving the concentrated powder of the drug 5 µg (5000 IU) in a chemically pure solution of sodium chloride (0.5%). Hence, the concentration effective on fish was observed, namely 0.5 µg (500 IU) per 1 cm³ (mL) of solvent. Females were injected with the drug "Surfagon" twice. The first injection contained 10% of the full dose of the drug, and the second injection contained 90% of the full dose of the drug. A time interval of 12 hours was maintained between the first and second injections of *S. lucioperca* females. Males were injected with the drug "Surfagon" once, simultaneously with the second injection of females. The dosage of the drug for males was 1 mL kg⁻¹.

In experimental group No. II, the drug "Vadilen" was used. It does not require prior preparation for injections, only compliance with the temperature regime and storage date. Since this drug is used for the first time to stimulate *S. lucioperca*, a dosage similar to that for sturgeon species was used. *S. lucioperca* females were injected with the drug "Vadilen" twice, at the concentration of 0.4 mg kg⁻¹; the first dose was 30% of the total and the second 70%. Zander males were injected once with the drug "Vadilen" at the concentration of 0.4 mg kg⁻¹, 12 hours after the first dose for females and simultaneously with the administration of their second dose (Kononenko et al 2016).

The egg fertilization was carried out by a semi-dry method. It was de-glued with dry milk, at the rate of 50 mg of milk per 1.5 kg of eggs (Davydov et al 1992). The fertilized caviar was incubated in a Weiss apparatus with a capacity of 16 L.

The volume of ejaculate was determined using an Eppendorf pipette-dispenser with an accuracy of 0.1 cm³. Sperm quality was determined by means of Zeiss Axiostar plus optical microscope with a 20x/0.40 phase-contrast lens, a JVC TK-C1480BE video camera, a Makler counting camera, and the Video Test Sperm 2.1 software from Video Test LLC. The use of the "Video Test Sperm 2.1" program for computer analysis of ejaculate (CASA) made it possible to obtain high accuracy of measurements and reliable and reproducible data in the analysis of sperm motility and morphological criteria.

Statistical processing of materials was performed using a package of standard Microsoft Office programs.

Results. Age 4-6 females with an average body weight of 2.4 to 2.6 kg were selected for the study. Age-5-6 males were selected with an average body weight of 1.7 to 2.2 kg. In general, males had lower average body weights than females, by 29.9% in the control group, by 33.5% in the experimental group I and by 12.0% in the experimental group II. A similar advantage in the values of female indicators over those of males was recorded in terms of length, height and girth of the body, which is quite characteristic of this species (Table 2).

Table 2
Characteristics of the brood fish used in the spawning campaign (mean±SE)

Indicators	Control group		Experimental group I		Experimental group II	
	♀(n=6)	♂(n=4)	♀(n=5)	♂(n=3)	♀(n=5)	♂(n=3)
Body weight, kg	2.4±0.20	1.7±0.05	2.6±0.15	1.73±0.05	2.48±0.21	2.2±0.25
Body length, cm	50.7±2.39	47.8±0.22	52.4±1.15	46.0±1.25	52.4±1.63	49.3±1.52
Body height, cm	16.6±0.53	15.3±0.41	17.4±0.43	15.0±0.47	17.2±0,58	17.0±0.47
Body girth, cm	37.8±1.74	32.7±0.24	40.0±0.99	33.0±0.47	40.4±2.44	35.2±0.76

At the same time, in males and females, the average condition factor was within 1.60-1.85. In the experimental group I, this parameter was the same in fish of both sexes. In the control group, the average condition factors in females were higher by 0.25 compared to males. In the experimental group II, as well as in the control group, females had a slight advantage (by 0.8) in this parameter. The body length to height ratio, with the exception of females from the experimental group II, exceeded 3 and was practically similar in individuals of both sexes from all experimental groups (Figure 1).

Despite the fact that females in experimental group I were characterized by slightly higher average values of body weight, they produced on average 1.9% fewer eggs compared to females from experimental group II. At the same time, females from the control group produced the fewest eggs (6.91±0.18). Females from experimental group I were characterized by smaller egg size compared to those of females from the control group and experimental group II. Accordingly, the number of eggs in 1 g was smaller by 35 and 20 eggs. In turn, this was reflected in the values of the working fertility, which

ranged from 192.6 ± 8.28 to 222.5 ± 16.48 thousand eggs. The variability index ranged from 8.6%, in females from the control group, to 14.81%, in females from the experimental group I (Table 3).

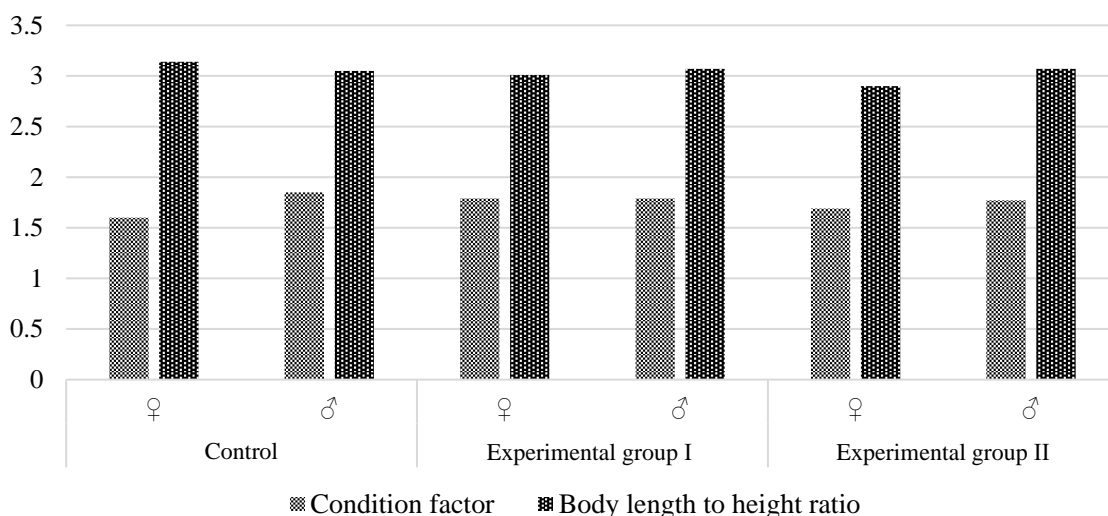


Figure 1. Condition factors and body length to height ratios of *Sander lucioperca* in experimental groups.

Table 3
Reproductive parameters of *Sander lucioperca* females (mean \pm SE)

Indicators	Control group (n=6)	Experimental group I (n=5)	Experimental group II (n=5)
Produced egg weight, g	177.50 \pm 7.40	198.75 \pm 14.62	202.50 \pm 17.81
The number of eggs in 1 g	1085,00 \pm 13.46	1120,00 \pm 12.75	1100,00 \pm 23.18
Working fertility, thousands eggs	192.6 \pm 8.28	222.5 \pm 16.48	221.4 \pm 16.23
Eggs obtained in % of body weight	6.91 \pm 0.18	7.41 \pm 0.15	7.84 \pm 0.11

The average value of the ejaculate volume from males in the experimental groups exceeded 1.5 mL and the highest – value, of 2.0 ± 0.25 mL, was found in the experimental group II. The working fertility of males from the experimental group II was 5.95 ± 0.36 million spermatozoa, 2.18% higher than in males from the control group and 4.53% higher than in males from the experimental group I. Males from the experimental group II were also characterized by a high value of the live spermatozoa number, of $90.33 \pm 1.91\%$. According to the values of this parameter, males from the experimental group II prevailed by 3.08% over males in the control group and by 1.0% over males from the experimental group I. Males from the experimental group I were characterized by the minimum values of the volume of ejaculate, of 1.63 ± 0.21 mL, with the maximum values of the variation of 22.54%. In males from the control group, the volume of ejaculate and the synchronicity of the obtained sexual products were characterized by a variability at the level of 14.57%, and were lower in comparison with the parameters of males from the experimental group II (Table 4).

Additional confirmation of the high reproductive qualities of *S. lucioperca* grown in aquaculture comes with the high values of the fertilization index, of more than 80.0% reaching their maximum, of 82.3%, in fish from the experimental group I. In fish from the control group, the values of the fertilization index were lower by 0.6% and by 3.7%, compared to those in fish from the experimental group I and from experimental group II, respectively (Figure 2).

Table 4

Reproductive parameters of *Sander lucioperca* males (mean±SE)

Indicators	Control group (n=4)	Experimental group I (n=3)	Experimental group II (n=3)
Volume of ejaculate, ml	1.78±0.13	1.63±0.21	2.0±0.25
The duration of active translational movement of spermatozoa, s	69.75±3.03	66.00±3.68	66.67±4.53
Working fertility, million specimens of spermatozoa	5.82±0.34	5.68±0.26	5.95±0.36
The number of live spermatozoa, from the total number, %	87.25±2.13	89.33±1.19	90.33±1.91

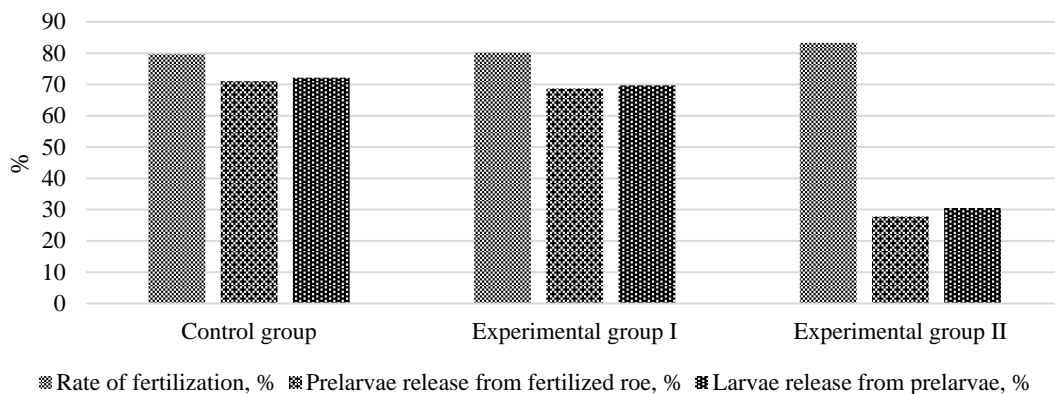


Figure 2. Indicators of egg fertilization and larval survival in the early stages of ontogenesis.

The prelarvae hatching from fertilized eggs also reached high values (71.01 ± 0.88 and 68.73 ± 0.75) in fish from the control group and experimental group I. In fish from the experimental group II, the value of this parameter was only 27.82 ± 0.89 . A similar situation was observed regarding the values of the yield of larvae from prelarvae.

Discussion. In many fish farms of Ukraine, during the factory reproduction, synthetic stimulators of Russian ("Nerestyn" drug series) and Hungarian ("Ovopel" drug series) manufacturers, as well as Ukrainian-made drugs "Surfagon" and "Vadilen" are used with the preparation of the pituitary gland of carp fish. All the above-mentioned preparations are characterized by the high power of a stimulating effect on breeders of carp fish species. However, the pricing policy and logistics capabilities of foreign products do not always meet the requirements of Ukrainian fish farms (Hrynzhevskiy et al 2006; Kovalenko et al 2015; Motlokh 2005; Mushyt 2017). Therefore, a decision was made to test domestically produced drugs, namely "Surfagon" and "Vadilen" in working with *S. lucioperca*.

During these studies, as a result of a two-time injection of the fish with the drug "Vadilen", the spawning behaviour was triggered 8-12 hours after the second injection. We determined that the use of the drug "Vadilen" ensures the production of egg from 100% of pike-perch females. The average value of the indicator of the produced egg weight was of 7.8% of the "Vadilen"-stimulated females' body weight. It is also worth noting that in the experimental group II, females produced sexual products most synchronously, which is confirmed by the level of the coefficient of variation, of 2.7%. Similar studies proved that the synthetic drug "Vadilen" has a reasonable result of stimulating effects on the broodstock of herbivorous fish, generally comparable to that of the preparation of the bream pituitary gland, which makes it possible to replace the latter with "Vadilen" (Shevchenko & Kovalenko 2018). It is considered promising to continue studying the effect of the drug "Vadilen" on the survival of *S. lucioperca* and the duration of exploitation of the brood stock.

The use of the drug "Surfagon" in working with *S. lucioperca* made it possible to establish that after 18-24 hours, 100% of sexually mature individuals enter the active spawning phase, by releasing sexual products. The average indicator of the produced egg weight was 7.4% of the weight of females. Males of this experimental group I had the shortest duration of active translational movement of spermatozoa, of 66.00 ± 3.68 s. This may be a consequence of the effect of the synthetic drug on the survival of spermatozoa, but confirmation is expected from additional studies. Certain sources from the literature indicate that the use of this drug was a negative experience in working with sturgeon, and its use on salmon indicates that the effect of this drug is manifested only on the 25th day, lasts 19 days and is observed in that part of the herd that swims against currents. The long-term effect of "Surphagon" is revealed later, 5 months after the injection, only in starving specimens, when their behaviour in the water flow is stabilized (Pavlov et al 2018).

The use of classical hypophysis in working with *S. lucioperca* is ineffective: females from the control group had the lowest rate of spawning and one of them did not produce sexual products at all. Also, the duration of the forward movement and the number of live sperm in males from the control group were lower than those in the experimental groups I and II. We can observe similar results in the work of Khrustalov (2021), which demonstrated attempts to use pike-perch pituitary gland as a gonadal-stimulating drug. In 30% of males, spermiation is possible after the first injection, in 35% after the second injection and in 35% after the third injection. In 40% of females, ovulation is possible after the first injection, in 40% after the second injection and in 20% after the third injection (Khrustalev 2021).

Conclusions. According to the results of the current research, the best results in working with pikeperch females was provided by using the chemical drug "Vadilen", which allowed obtaining an egg quantity of 7.8% of the female weight. The drug "Surfagon" was the second most effective for working with pikeperch females. It allowed obtaining an egg quantity of 7.4% of the female weight. The lowest egg quantity results (6.9% of the weight of the female) were registered with the pituitary preparation. At the same time, explaining the low values of the indicators of the release of prelarvae from the fertilized eggs (27.8%) and of the release of larvae from the prelarvae (30.5%) requires further research. In particular, it is worth calculating the economic feasibility of using the above drugs. In addition, it is necessary to analyse the phenomenon of the presence of females that did not have an adequate response to stimulation with the three above-mentioned drug injections: either they did not give sexual products or they died during the final stages of the spawning campaign. In general, the obtained results allow to state that the most effective for ensuring high values for the indicators of controlled *S. lucioperca* spawning, even during the off-season, are the preparations "Vadilen" and "Surfagon". In turn, it is most rational to use these drugs in recirculation aquaculture systems, pool fish farms or hatcheries departments.

Conflict of interest. The authors declare no conflict of interest.

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