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BIOCHEMISTRY

# Long-Term Influence of Surfagon Injection on the Cytological Condition of the Gonads and Level of Thyroid and Sexual Steroid Hormones in Young Brown Trout *Salmo trutta*

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**Abstract**—The long-term effect of the injection of a synthetic analogue of gonadotropin releasing hormone—Surfagon—on the reproductive system of juvenile brown trout *Salmo trutta* has been studied. The concentration of thyroid hormones in the blood, the rate of gametogenesis, and physiological adaptation of individuals to external conditions increased over five months after injection. The changes identified predetermine the formation of a predominantly resident fish of the brown trout population. Based on the patterns found, the interrelation of the hormonal status, puberty, and the formation of life strategies in salmon species has been analyzed.

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## INTRODUCTION

The fish reproductive system, its functioning, and development are largely related to the hormonal regulation. At different stages of ontogenesis, gonadotropin-releasing hormone (GnRH) and thyroid (Cyr and Eales, 1996; Comeau and Campana, 2006; Nelson et al., 2010) and sex steroid hormones regulate the formation and maturation of the gonads. GnRH is one of the most important hormones of the hypothalamus with a wide functional spectrum of action in the organism (Chen and Fernald, 2006, 2008). Its main function is the formation and development of the gonads by regulating the synthesis of follicle-stimulating and luteinizing hormones (Crim et al., 1983, 1986; Makeeva, 1992; Maugars and Schmitz, 2008). GnRH and its analogues are used in aquaculture to stimulate the maturation of reproductive cells in various fish species (Zohar and Mylonas, 2001; Chebanov et al., 2004) and synchronize their spawning (Mylonas et al., 1992; Powell et al., 1998).

Synthetic peptide Surfagon is GnRH analogue with high specific activity, ensuring its stronger biological effect. Previously, we studied (Pavlov et al., 2018a, 2018b) the effect of Surfagon on the state of gonads in juvenile rainbow trout *Parasalmo mykiss* (=*Oncorhynchus mykiss*). For the first time, it was shown that the introduction of this drug not only increases the rate of gametogenesis, but also reduces the number of gonad abnormalities. On the basis of cytomorphological results and data from behavioral experiments (Pavlov et al., 2016), it was shown that Surfagon enhances the physiological adaptation of fish, i.e., adaptation to the conditions of the experiment, and reduces their motivation for migration activity.

For a short time (several hours), Surfagon increases the concentration of gonadotropins in the organism (*Opisanie*..., 2004). Drug effects were recorded on 30–40 days after injection. We did not find published data on the longevity of the effect from a Surfagon injection on the reproductive system of fish, although the interrelations of fish puberty and the formation of their life strategies have been repeatedly mentioned in various literature sources (Gruzdeva et al., 2013, 2017; Pavlov et al., 2014; Pavlov et al., 2015). The long-term effect of Surfagon on the formation of fish life strategies has not previously been evaluated.

The aim of this work is to study the cytological state of the gonads and the concentration of thyroid and sex steroid hormones in the blood of the brown trout five months after the injection of Surfagon and to evaluate the possible role of these indicators in formation of their life strategy.

## MATERIALS AND METHODS

The experiment was conducted on juvenile brown trout of the age of 10–15 months in the Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences. Fish at the age of six months were brought from the hatchery Yanisyarvi LTD (Republic of Karelia). Juveniles were kept in pools 2.5 × 0.5 m with a closed water supply system, water level approximately

0.5 m, average stocking density of 320 ind./$m^3$, water temperature 13–15°C, flow rates in pools was 1 cm/s, illumination of 3000–7000 lx. The fish were fed two times a day with AQUAREX Forel' Start 55/13 artificial granulated food (Russian Federation) with a granule size of 1.2–1.4 mm. The average length and weight of fish at the age of 10 months were 6.0 cm and 16.6 g, respectively.

Before injection, the test specimens were kept for 1–2 min in a lidocaine anesthetic solution (100–150 mg/L of water). An indicator of its effect was discerned by the decreasing locomotor activity of the fish. Surfagon in a sodium chloride solution was injected to experiment individuals at the age of ten months by an insulin syringe intramuscularly under the pectoral fin. To enhance the hormonal effect, the injection was performed twice (Chebanov et al., 2004) with a 48-hour interval. The concentration of Surfagon was 10 μg/kg for the first injection and 15 μg/kg of fish for the second one. The dosage of Surfagon was in the range of action used previously on rainbow trout (Pavlov et al., 2016; Pavlov et al., 2018a, 2018b). Test and control (intact) individuals, with 80 ind. in each group, were placed immediately after the injection into two separate tanks (1.2 × 0.5 m) with a water level of 0.5 m approx. Under such conditions, the fish were kept for five months until the age of 15 months.

Previously conducted special experiments showed (Pavlov et al., 2019) that the reaction of fish to handling stress was not recorded already on the seventh day after the drug injection. Therefore, the selected long-term observation periods allow not to inject the control group with physiological solution (placebo).

The degree of brown trout smoltification was evaluated by the development of silver body coloration according to a scale (Kalinina, 2012) modified by us for brown trout: 0, there is no silvery coloration of the scales; I, silvery coloration is observed only on the dorsal surface of the body at the head part; II, silvering extends to the lateral parts; III, silvering extends to the ventral part of the body, IV, silvery coloration covers more than half of the body surface.

The body length ($L$) and body weight ($W$) were measured for the test and control fish at the age of 15 months (five months after the injection of Surfagon). After the test and control juveniles reached the age of 15 months, their blood was sampled to determine the concentration of hormones and their gonad fragments, to evaluate the cytological state of the gonads. Samples were taken from 73 individuals at 15 months of age: 33 control individuals and 40 test ones.

Gonads were fixed in Bouin's fluid to evaluate their cytological state. Histological preparations were made according to standard methods (Mikodina et al., 2009) using semi-automatic specialized histological equipment (Medite, Germany): the tissue processing system TPC-15, the paraffin embedding system TES-99, and the Meditome M530 microtome. Sections 5 μm thick were successively stained with Ehrlich's hematoxylin and a water–alcohol solution of eosin using specialized series for staining preparations. Photos of sections of gonads were obtained using a Biorevo BZ-9000 motorized microscope (Keyence, Japan).

Blood was sampled with a 1 $mL^3$ syringe from the fish caudal vein. The brown trout blood was centrifuged for 5 min at 5000 rpm. The resulting plasma was poured into sterile tubes and stored in a freezer at −30...−20°C. The concentration of thyroxine ($T_4$), triiodothyronine ($T_3$), testosterone, and estradiol-17β were determined by enzyme-linked immunosorbent assay with kits (DRG, Germany) on an MR 96A device (Mindray, China). The ratio of thyroid hormones ($T_4/T_3$) was calculated to determine the dependent dynamics of their content in the blood, since it is known that due to the process of deiodination, $T_4$ is converted into $T_3$ (Cyr and Eales, 1996; Hulbert, 2000).

Cytological changes in the gonad were assessed by the gonad maturity stage, the presence and number of abnormalities in the structure, and the calculated parameters of the gametogenesis rate. The nuclear–cytoplasmic ratio (N/C) was used to determine the rate of oogenesis. It was calculated as the ratio of the size of the nucleus to the size of the cytoplasmic material in the oocyte at a section near the central part of the oocyte (Pavlov et al., 2014). The smaller N/C ratio corresponds to the larger size of the oocyte. More than 725 oocytes were measured in the test and control individuals.

The state of testes was determined by the number of different types of germ cells (spermatogonia, spermatocytes, and spermatids) per unit area of the testicular section (10000 $\mu m^2$). The ratio of the area occupied by mature cells (spermatozoa) to the total area covered by the microscope objective of the section area was calculated.

Measurements of oocytes for the calculations of the N/C ratio and the number of cells per section of the testes were performed using the ImageJ 1.51k software. Statistical processing was performed using Student's $t$-test, the Mann–Whitney U-test, and the analysis of variance.

## RESULTS

At the age of 15 months, fish in both groups begin smoltification. The degree of smoltification in juveniles in the test group was lower ($p < 0.01$ by the Mann–Whitney U-test) than in the control group (Fig. 1).

The length and weight of juvenile brown trout at the age of 15 months in the test group were 16.1 ± 0.36 (11.9–21.2) cm and 36.3 ± 2.19 (14.2–65.7) g, respectively, and in the control group, 17.2 ± 0.34 (14.3–22.3) cm and 42.0 ± 2.88 (20.7–99.4) g, respectively. (Hereinafter, before the parentheses we present the

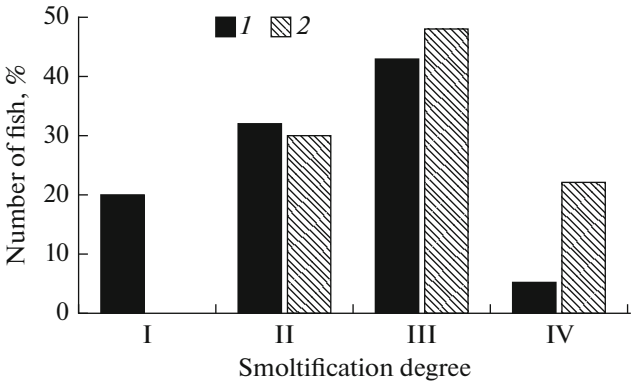


**Fig. 1.** Distribution of the test (*1*) and control (*2*) juveniles of the brown trout according to the degree of smoltification. The total number of fish in the test group and the total number of individuals in the control group were taken as 100%.

average value of the indicator and its error; in parentheses, the limits of variation of the indicator.) The test individuals were shorter ($p < 0.05$, Student's *t*-test) than the control ones.

Analysis of variance showed that the degree of smoltification is related to the length and body weight of individuals ($p < 0.001$): a high degree of smoltification was observed in fish with larger body sizes.

### *Cytological State of the Gonads of Juvenile Brown Trout*

The gonads of the test and control females of the brown trout differ only in their estimated indicators (N/C ration and the proportion of the structure anomalies). The testes in the two groups of fish differ in maturity stages and estimated indicators (the ratio of cell types to the area, the ratio of the area of sperm cells to the total area of the section). The description of the cellular structure of the gonads for the same maturity stages is given without division into the test and control groups of fish.

#### *Females*

The ovaries of the test and control fish were at maturity stage II. Sex cells were represented by oocytes of the previtellogenesis period; their diameter varied from 40 to 260 microns. Cells with a size of ~100 μm were the most numerous in the gonads. In oocytes with a diameter of 40–60 μm, cytoplasmic growth has just begun, and in the largest cells (with a diameter of 160–240 μm), this process has already been completed (Fig. 2a). In the cytoplasm of small and medium oocytes, zones of localization of organelles containing RNA, intensively stained with hematoxylin, are clearly visible. Zones of RNA accumulation are either arranged in an orderly manner, forming a ring around the cell nucleus (Fig. 2b), or lie chaotically in the cytoplasm (Fig. 2c). RNA zones are no longer observed in the large cells (Fig. 2d).

In the ovaries of the fish from both groups, a significant number of dead gametes were found. In the gonads of the fish treated with Surfagon, the proportion of such cells in some individuals reaches 27.5%; the average value for the group was 15%. In the ovaries of the control fish, dead cells were found frequently: in some individuals, they reach 46% of the total number of oocytes; on average in the group, 28%.

The ratio N/C of oocytes in the gonads of the experimental and control individuals was $0.21 \pm 0.003$ (0.09–0.52) and $0.25 \pm 0.005$ (0.11–0.66), respectively. Oocytes in the test fish were larger in comparison to the control group ($p < 0.001$, Student's *t*-test). Thus, 55% of oocytes in the gonads of the experimental group have $N/C < 0.2$, while in the control group it was observed in 31% (Fig. 3). Some oocytes of the test individuals were characterized by $N/C < 0.1$. In the gonads of the control group, oocytes of the early stage of cytoplasmic growth with $N/C > 0.6$ were registered.

#### *Males*

The testes of 95% of fish from the test group were at the maturity stages II and III. The gonads of one individual of the test group were at maturity stage V (Fig. 4). In the control group, approximately 75% of males had gonads of maturity stages II and III. In five specimens, the gonads were more developed; they belonged to stages IV and V.

The sex cells of maturity stage II are located in cysts; they are represented by numerous spermatogonia and a small number of smaller cells starting the meiosis prophase, first-order spermatocytes (Fig. 5a). The calculation method has shown that the number of spermatogonia per unit area (1 $mm^2$) in the gonads of individuals of the test group is noticeably higher ($p < 0.05$, Mann– Whitney U-test) than in the testes of the control ones: 9423 (5766–12643) items vs. 7400 (5896–10690) items, respectively.

Spermatogonia of the third maturity stage of the gonads are not abundant; spermatocytes of the first and second orders predominate in the testes (Fig. 5b). Spermatocytes of the second order are 1.5–2 times smaller than those of the first one; they are more intensely stained with hematoxylin. The testes of some fish develop more intensively (late maturity stage III), spermatogenesis is actively occurring in them: seminiferous tubules with spermatids and spermatozoa developed (Fig. 5c). The number of spermatogonia per unit area (1 $mm^2$) in the gonads of the test fish group is much smaller ($p < 0.05$, Mann–Whitney U-test) than in the testes of the control fish group: 534 (88–1442) vs. 912 (403–1720), respectively. The number of spermatocytes and spermatids in the gonads of the fish from the two groups are almost the same. The

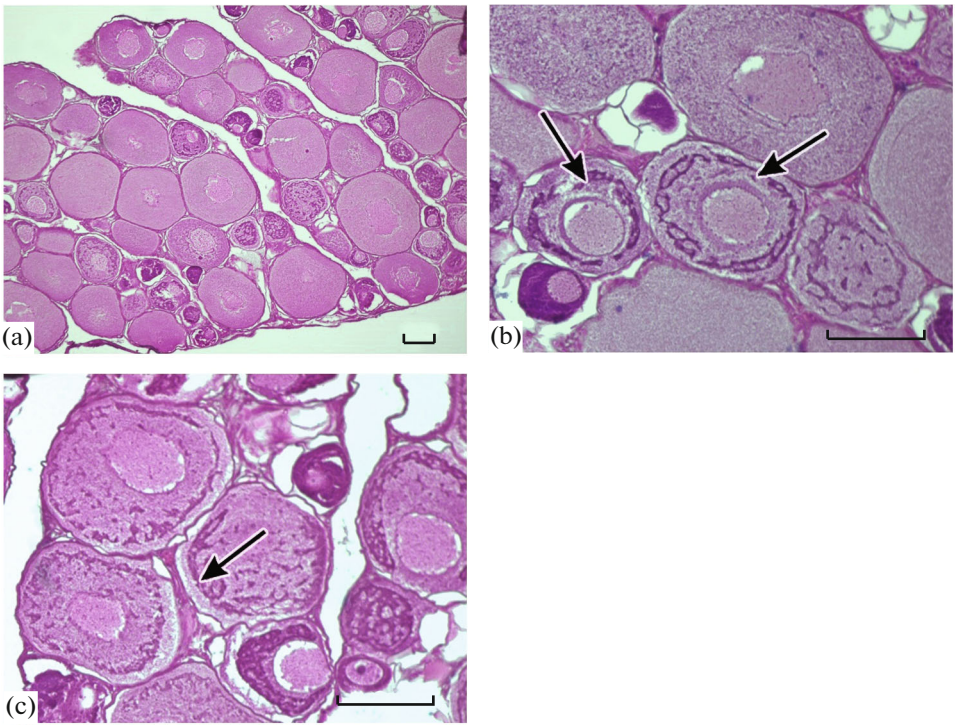


**Fig. 2.** Ovaries of the juvenile brown trout of maturity stage II. (a) Oocytes of the previtellogenesis period, general appearance; (b) ring zones of RNA localization (arrows); (c) RNA localization zones are randomly located in the cytoplasm (arrow). Scale: 100 (a) and 20 microns (b, c).

spermatozoa of the most developed gonads also occupy similar areas.

The sexual products finally mature in the gonads of maturity stage IV. Testes contain spermatids and spermatozoa which are formed as a result of the second meiotic division. Spermatozoa are more intensely stained with hematoxylin in blue–violet in comparison to the spermatids. Still, there are areas of generative tissue containing spermatocytes of the first and second orders and single spermatogonia (Fig. 5d).

In maturity stage V, seminiferous tubules increase in size and are completely filled with spermatozoa (Fig. 5e).

One hermaphrodite was found in the control group (Fig. 6). Spermatogonia and single oocytes of the previtellogenesis period were present in the gonads of this

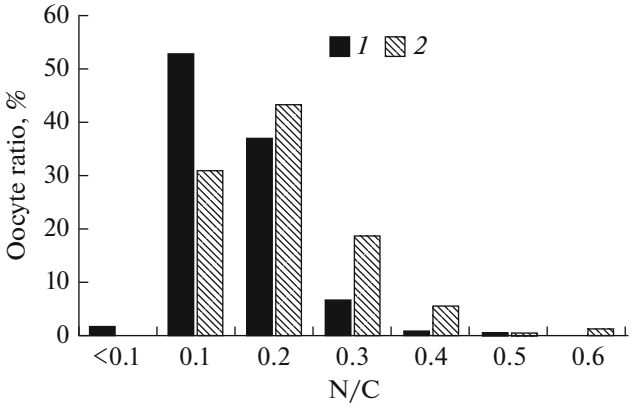


**Fig. 3.** Distribution of oocytes by the nuclear-cytoplasmic ratio (N/C) in the gonads of test (*1*) and control (*2*) juvenile brown trout. The total number of oocytes in the gonads of the test group and the total number of oocytes in the gonads of the control group were taken as 100%.

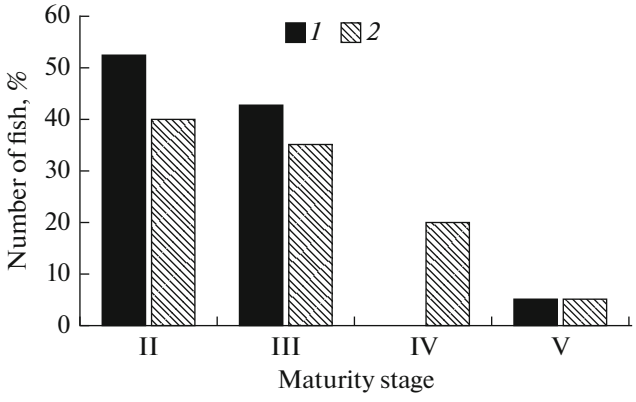


**Fig. 4.** Distribution of males of the test (*1*) and control (*2*) groups of the brown trout according to the maturity stages of their gonads. The total number of males in the test group and the total number of males in the control group were taken as 100%.

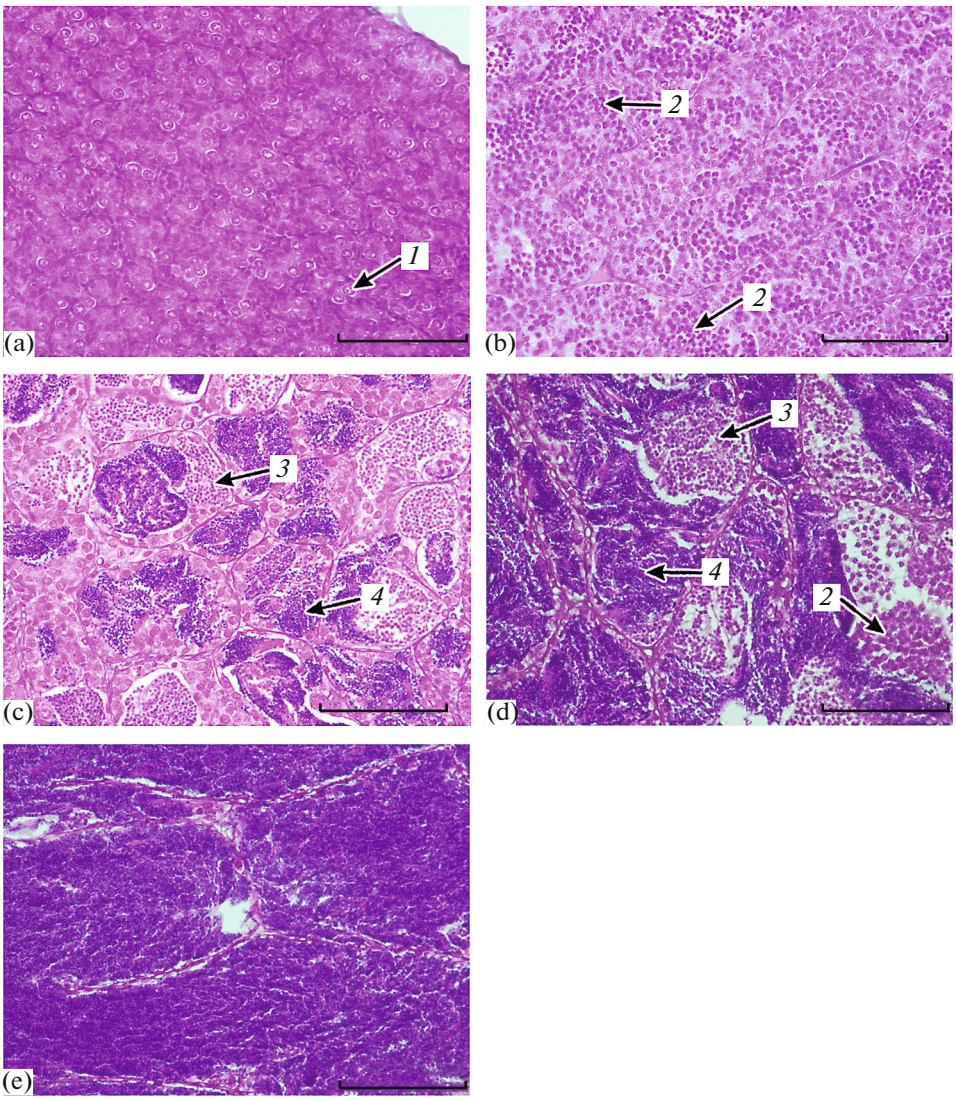


**Fig. 5.** Cytological structure of the testes in juveniles of the brown trout at the age of 15 months. (a) Cysts with spermatogonia (maturity stage II); (b) spermatocytes of the first order (maturity stage III); (c) seminiferous tubule formation (maturity stage III-late); (d) seminiferous tubules with spermatids and spermatozoa (maturity stage IV); (e) seminiferous tubules filled with spermatozoa (maturity stage V). *1*, Spermatogonia; *2*, spermatocytes; *3*, spermatids; *4*, spermatozoa. Scale: 100 microns.

individual. Cavities are localized instead of dead oocytes in the generative tissue. In some parts of the gonad, connective tissue hypertrophy was observed.

The presence of single hermaphrodites in salmon fish populations is not uncommon (Makeeva, 1992; Mikodina and Pukova, 2002; Pavlov et al., 2013). The presence of one hermaphrodite in the group does not indicate significant deviations in the development of the sex glands of the control fish.

### *Concentration of Thyroid and Sex Steroid Hormones in the Blood of Brown Trout*

An analysis of variance showed that the content of thyroid hormones in the blood is associated with the individual belonging to the test or control group ($p < 0.05$) and the degree of its smoltification ($p < 0.05$). The higher degree of fish smoltification was in compliance with the lower concentration of thyroid hormones in its blood. The concentration of $T_3$ in the blood also depends on the sex of the individual ($p < 0.05$).

The concentration of $T_3$ in the blood of females of the control group was significantly higher ($p < 0.05$, Student's $t$-test) than in males: 16.4 ± 1.66 (5.4–30.4) and 11.9 ± 1.2 (1.5–21.6) ng/mL, respectively. In the experimental group, the $T_3$ concentration in females was 19.6 ± 1.95 (5.8–40.2) ng/mL. The value of this indicator is higher ($p < 0.05$ by Student's $t$-test) in the blood of males from the experimental group in comparison to the males from the control group (Fig. 7a).

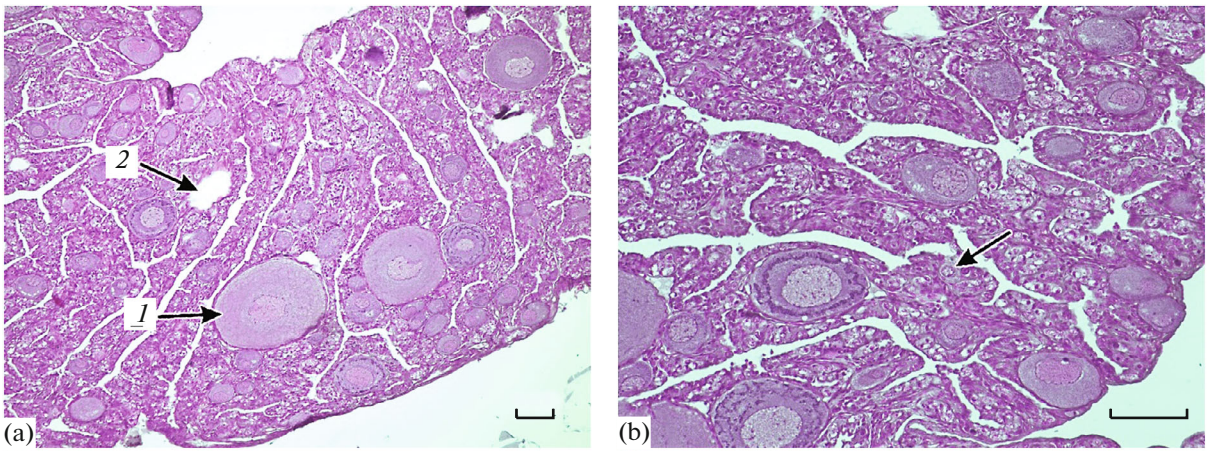


**Fig. 6.** Cytological structure of the gonads of a hermaphrodite from the control group of the brown trout at the age of 15 months. (a) General view of the gonad of a hermaphrodite, oocytes of the previtellogenesis period (*1*) and a cavity (*2*) in the place of a dead oocyte; (b) germination of connective tissue, cysts with spermatogonia (arrow). Scale: 100 microns.

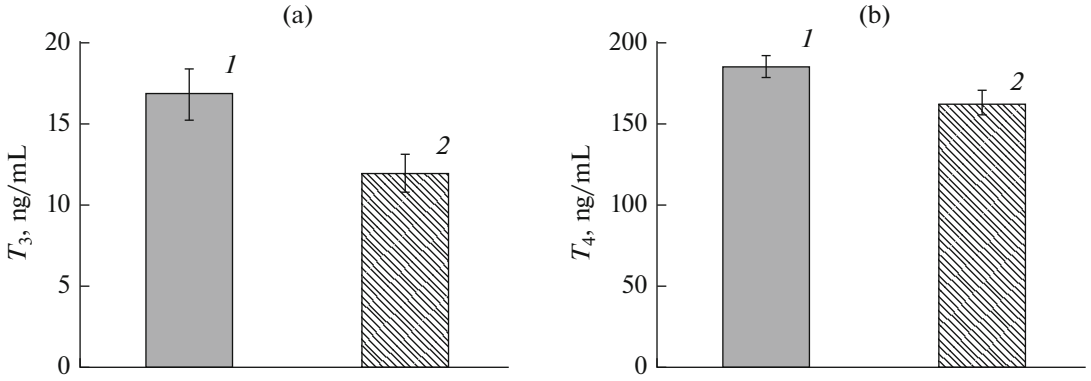


**Fig. 7.** Concentration of thyroid hormones in the blood of the test (*1*) and control (*2*) groups of brown trout. (a) Concentration of $T_3$ in males; (b) total concentration of $T_4$ in females and males.

The concentration of $T_4$ in the blood of fish from the test group was higher ($p < 0.05$, Student's *t*-test) than in individuals from the control group (Fig. 7b). (The graphs are given only for significantly different indicators in the test and control. The values of $T_4$ concentrations are given without division into females and males, since according to the analysis of variance, this factor does not depend on the sex of the fish ($p > 0.05$).)

The ratio $T_4/T_3$ does not differ in females and males and does not depend on belonging to the test or control groups. Its average value is $12.4 \pm 1.02$ (1.1–44.4) and $17.4 \pm 4.4$ (3.8–171.8), respectively.

According to the analysis of variance, the concentration of estradiol-17β in the blood of the brown trout depends only on their degree of smoltification ($p < 0.05$). With increasing degree of smoltification, the concentration of the hormone decreases. The testosterone concentration in the blood depends on the maturity stage of the gonads and the sex of the fish ($p < 0.001$). The quicker the testes develop, the higher the testosterone concentration in the blood. The testosterone concentration is higher in the blood of males compared with females both in the test group and in the control (Student's *t*-test, $p < 0.01$ and $p < 0.001$, respectively) (Table 1).

## DISCUSSION

The obtained data indicate that Surfagon has a long-term effect on the development of the reproductive system of juveniles of the brown trout. The effect of the drug injection is observed five months later at both the cytomorphological and hormonal levels. Earlier (Pavlov et al., 2018a, 2018b), we assumed that Surfagon acts as a signaling factor leading to a rearrangement of the internal hormonal regulation of the recipient. Our results confirm the hypothesis.

Let us consider the consequences of the effect of Surfagon on some morphological (degree of smoltification, cytomorphology of the sex glands) and biochem-

**Table 1.** Concentration of hormones in the blood of juveniles of brown trout in the test and control groups

| Hormone | Test | | Control | |
|---|---|---|---|---|
| | ♀ | ♂ | ♀ | ♂ |
| Estradiol-17β, pg/mL | 1.5 ± 0.15<br>0.6–3 | 1.8 ± 0.33<br>0.9–9 | 1.5 ± 0.14<br>0.8–3.1 | 1.8 ± 0.27<br>0.7–6.5 |
| Testosterone, ng/mL | 4.4 ± 0.54<br>0.3–10.4 | 9.1 ± 1.15<br>3.3–24.5 | 4.1 ± 0.5<br>1.5–9.6 | 10.7 ± 1.31<br>1.9–22.1 |

Above the line is the average value of the factor and its error; below the line, the limits of variation of the factor.

ical indices associated with the development of the reproductive system of juveniles of the brown trout.

The effect of Surfagon on the gonads of females is expressed in the acceleration of oogenesis (reduction of the N/C ratio) and an increase in the development rate of the oocytes. According to published data (Crim et al., 1983, 1986), the acceleration of oogenesis (an increase in the gonadosomatic index, an increase in the weight and diameter of oocytes) under the influence of other synthetic analogues of GnRH was also observed in the Atlantic salmon *Salmo salar*. The action of the drug results in a decrease in the number of cells in the destruction state, and the proportion of anomalies in the generative tissue decreases. Earlier, on the example of the rainbow trout (Pavlov et al., 2018a, 2018b), we showed that 30–40 days after the injection of the drug gametogenesis in fish stabilizes and the proportion of destroyed oocytes of the previtellogenesis period decreases.

Unlike the ovaries, the testes of the brown trout of both the test and the control groups were at different maturity stages. In the test group, >90% of males had gonads of maturity stages II and III. In the control group, there were males with the gonads of maturity stages II (40%), III (35%), IV (20%), and V (5%). Consequently, a quarter of the control males had almost mature testes; i.e., they had reached puberty at least a year earlier. The differences between the test and the control groups of fish in the rate of spermatogenesis in the gonads of maturity stages II and III could be determined only by calculated methods. The number of spermatogonia (per unit area) was higher in testes of maturity stage II in the test group ($p < 0.05$) in comparison to the gonads of the same stage of the control males. This indicates a more intensive spermatogenesis, accelerated reproduction of spermatogonia, in the gonads of the test group compared with the control group. The testes of individuals of the test group at the third maturity stage, in contrast, contained fewer ($p < 0.05$) spermatogonia than the gonads of fish from the control group. It is known (Crim et al., 1983) that the effect of the GnRH analogue on the testes of Atlantic salmon leads to an increase in the proportion of spermatids and spermatozoa against the background of a decrease in the number of spermatocytes. In our experiments, the number of cells of the late maturity stage in the gonads of the test group did not increase in comparison to the control group. However, we consider a decrease in the proportion of cells of the early stage (spermatogonia) in the test individuals with gonads of maturity stage III as a tendency to an increase in the rate of spermatogenesis in the test group in comparison to the control group.

Surfagon injection results in an increase in the concentration of thyroid hormones in the blood of fish from the test group compared with individuals from the control group: the concentration of $T_4$ in the blood increased both in females and males, and the concentration of $T_3$ increased in the males of the test group. The using of GnRH analogs on Sockeye samon *Oncorhynchus nerka* at the age of 2+ also led to an increase in the $T_3$ concentration and a slight increase in the $T_4$ concentration in the blood (Plate et al., 2002). The ratio $T_4/T_3$ does not differ in the studied groups, which indicates the absence of a pronounced effect of the Surfagon on the deiodination process. This is consistent with the literature data (Plate et al., 2002), indicating that there is no such relationship between the other GnRH analogue used and the deiodination process in Sockeye salmon *O. nerka*.

The concentration of sex steroid hormones in the blood of the experimental and control groups did not differ. Within groups, males were characterized by a higher content of testosterone in the blood than females. The results obtained for sex steroid hormones indicate that the action of the Surfagon poorly affects the synthesis of sex steroid hormones in juvenile brown trout.

As noted above, thyroid hormones perform not only their main energy function, but also, together with sex steroid hormones, participate in the development of the reproductive system. Thyroid hormones in synergy with gonadotropins can stimulate the development of gonads in females (Cyr and Eales, 1996). An increase in the thyroxin concentration was observed in freshwater in juveniles of the Black Sea salmon *S. trutta labrax* with intensive gametogenesis in the gonads (Pavlov et al., 2014). Most likely, the observed acceleration in the development of the gonads of the test group in the early stages of ontogen-

esis occurs due to an increase in the activity of the thyroid axis.

The influence of Surfagon on the increase of the physiological adaptations of fish to external conditions was shown earlier (Pavlov et al., 2018a, 2018b). Under adverse conditions, the proportion of structural anomalies in the gonads decreases. At the same time, the proportion of fish moving downstream searching for more suitable habitats decreased (Pavlov et al., 2016; Pavlov et al., 2018a).

Many authors (Hoar, 1959; Sage, 1973; Peter and Peter, 2011; Dolomatov et al., 2013) suggest that one of the main functions of thyroid hormones is the adaptation of an individual to the environmental conditions. In particular, thyroid hormones increase the adaptation of fish to temperature fluctuations of water during seasonal changes (Hoar, 1959). It was shown (Arjona et al., 2011) that thyroid hormones together with corticosteroids regulate the adaptive rearrangement of ion exchange during fish smoltification by changing the activity of $Na^+$,$K^+$-ATPase. We have found (Pavlov et al., 2018a) that a preliminary injection of Surfagon into juvenile rainbow trout kept for four days at a high water temperature reduced the anomalies in the development of its gonads. There is good reason to believe that the increase in physiological adaptation to the conditions of housing in the test fish group occurs due to an increase in the synthesis of thyroid hormones catalyzed by the injection of the drug.

Assumptions about the relationship of the reproductive system and the trajectory of fish development were formulated earlier (Thorpe, 1994; Pavlov and Savvaitova, 2008; Olsson and Greenberg, 2011; etc.); specific data were also presented on the relationship between the cytomorphology of fish gonads and their life strategy (Gruzdeva et al., 2013, 2017; Pavlov et al., 2014). Published data indicate that the accelerated development of the gonads in fish is associated with a resident way of life, and slowed down, with anadromous. It is known (Yamamoto et al., 1997; Volkoff and Peter, 1999; White et al., 2002; Yaron and Levavi-Sivan, 2011) that the main function of GnRH is the formation and development of the gonads. As was shown in previously conducted behavioral experiments (Pavlov et al., 2016), Surfagon reduces the proportion of fish moving downstream. Apparently, the injection of Surfagon switches the hormonal regulation of the body towards acceleration of puberty, blocking the implementation of downstream migration in individuals (potentially resident fish).

Let us consider smoltification of juveniles of brown trout and the associated physiological changes. By the age of 15 months, juveniles of brown trout, both in the test and control groups are smoltificated to different degrees. Smoltification is typical for the brown trout in its natural habitat and is associated with the formation of different life strategies in fish within the population, with the formation of anadromous, resident, and transitive forms. (In this paper, the term form is understood as "neutral" according to Mayr (Mayr, 1953) and is not associated with the formation of any genetic differences.) Smoltification of the fish indicates a tendency to anadramous life strategy, realization of which forces them to run to the sea for the feeding period (Bone and Moore 2008; Jonsson, B. and Jonsson, N., 2011). For some individuals of the control and test groups, silvering of the body is a temporary sign of morphological changes. Such fish would eventually undergo desmoltification. Salmon desmoltification is frequent due to a number of both external and internal factors. It leads to the loss of silvery color of individuals, a decrease in the activity of $Na^+$,$K^+$-ATPases, and the reverse adaptation of fish to fresh water (Jonsson, B. and Jonsson, N., 2011).

Precocious males with gonads of maturity stages IV–V by the age of 15 months actually formed a separate form. It should be noted that precocious males was less common in the test group than in the control one (5% vs. 25%). By a number of researchers (Bohlin et al., 1994; Fleming, 1996; Metcalfe, 1998; Morgan and Metcalfe, 2001), the formation of precocious salmon fishes is associated with favorable environmental conditions in early ontogenesis. It can be assumed that the Surfagon injection due to switching of the resources of the organism to the rapid development of the gonads leads to the formation of a predominantly resident form of fish, but not to their early maturation.

The degree of smoltification of juveniles of brown trout differs in the test and control groups. The silvery color of the body is less pronounced in the test individuals. The increase in the proportion of anadromous fish in the population is usually caused by unfavorable environmental conditions, in particular, a lack of food resources (Pavlov et al., 2001, 2008; Pavlov and Savvaitova, 2008; Wysujack et al., 2008). We assumed (Pavlov et al., 2016; Pavlov et al., 2018a) that the injection of Surfagon, by increasing the physiological adaptation of individuals to existing environmental conditions, reduces their motivation to implement downstream migration; i.e., it changes the development trajectory of fish towards residency. The revealed slow-down of smoltification in test fish is consistent with this hypothesis.

The analysis of variance showed that the degree of smoltification of juveniles of the brown trout is associated with the majority of the indicators considered in this work. Here is the consideration of such relationships obtained both for brown trout juveniles and for other fish species:

—The greater length and weight of the brown trout was in compliance with the higher degree of its smoltification. Similar results were obtained on the juveniles of the Black Sea salmon (Pavlov et al., 2010, 2012): individuals with an anadromous life strategy were characterized by larger body sizes.

—The concentration of thyroid hormones in the fish blood decreases at the later stages of trout smoltification. It is known (Jonsson, B. and Jonsson, N., 2011) that thyroid hormones take a primary part in the silvering process during salmon smoltification. Our data on thyroxin coincide with the results of Dickhoff et al. (Dikhoff et al., 1978). The concentration of thyroxin in Coho salmon *O. kisutch* increased before the process of smoltification and decreased to almost the initial values by the end of the process.

—The concentration of estradiol-17β in the blood decreases during the passing of the smoltification process in the juvenile brown trout. At the age of 15 months, oogenesis proceeds more intensively in the resident form of the Black Sea salmon in comparison to the anadromous form (Pavlov et al., 2014). Accordingly, a decrease in the concentration of estradiol-17β in the blood may be determined by a change in the oogenesis rate.

Thus, the injection of Surfagon into juveniles of the brown trout has a prolonged effect (at least five months) not only on the reproductive system of the fish, but also on their development trajectory, on the formation of life strategies. By increasing the synthesis of thyroid hormones, Surfagon increases the rate of gametogenesis, enhances the physiological adaptation of individuals to environmental conditions, and stimulates the formation of predominantly resident forms of brown trout. Smoltification of fish is related to their size and the concentration of thyroid hormones and estradiol-17β in the blood. We assume that the drug injection results in an increase in the concentration of gonadotropins at the early stages of ontogenesis that can regulate the trajectory of development of the brown trout by increasing the rate of gametogenesis. Similar changes in the gonadotropin concentration in the organism can occur in fish and in their natural habitat under the influence of temperature, photoperiod, dietary habits, gender of the individual, and degree of maturity of its gonads (Peter, 1981; Hellqvist et al., 2006; Martyniuk et al., 2009). The results obtained indicate the interrelation of four processes that occur simultaneously in the fish: hormonal regulation, growth, puberty, and formation of a life strategy.

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## COMPLIANCE WITH ETHICAL STANDARDS

*Conflict of interests*. The authors declare that they have no conflict of interest.

*Statement on the welfare of animals*. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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