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Surfagon Effect on Rheoreaction of Young Brown Trout *Salmo trutta*

D. S. Pavlov[a], E. D. Pavlov[a, *], V. V. Kostin[a], P. I. Zaripov[a], and M. A. Ruchiev[a]
[a]*Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, 119071 Russia*
**e-mail: p-a-v@nxt.ru*
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Abstract—The effect of surfagon injection (synthetic analog of gonadotropic-releasing hormone) on the kind and time of rheoreaction change in juvenile brown trout *Salmo trutta* is studied. In spite of the fact that the increase of gonadotropins in the organism after surfagon injection is recorded just during several hours, the action of this preparation on the rheoreaction is discovered only at the 25th day, continues for 19 days, and is seen as the increase of the part of fish swimming upstream. The long-term effect of surfagon is found later – 5 months after the injection. This effect is present only in starving specimens and is the stabilization of their behavior in the water flow.

Keywords: brown trout *Salmo trutta*, behavior in water flow, rheoreaction, starvation, surfagon, gonadotropic-releasing hormone, hormonal imprinting

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The rheoreaction of fish is inherent and influences their behavior in the water flow. Depending on direction of movement in relation to the water flow, the fish manifest three types of rheoreaction: positive, negative, and compensatory. The ratio of these types reflects the motivational component of the migratory activity of fish in the water flow (Pavlov et al., 2010). Both the rheoreaction of fish and their migratory activity are connected in many ways with the individual hormonal state (Boula et al., 2002; Jonsson and Jonsson, 2011; Pavlov et al., 2014).

The gonadotropic-releasing hormone (GnRH) is one of the major hormones of the hypothalamus possessing a wide functional spectrum of action on the organism (Chen and Fernald, 2006, 2008). Its main function is related to formation and development of gonads. GnRH also controls the spawning behavior (Yamamoto et al., 1997; Volkoff and Peter, 1999; White et al., 2002), aggressive behavior of fish (Soma et al., 1996; Ogawa et al., 2006), and their behavior in the water flow (Plate et al., 2003).

The surfagon is a synthetic analog of GnRH but is more active and more strongly influences the gonadotropic function of the hypophysis. Surfagon and other analogs of GnRH are applied in aquaculture for stimulation of gonad maturation in various fish species. Previously, the effect of surfagon on the rheoreaction of juvenile rainbow trout *Parasalmo mykiss* (=*Oncorhynchus mykiss*) was studied (Pavlov D. et al., 2016). It was shown for the first time that the injected specimens prefer upstream swimming 30–40 days after surfagon application, while intact specimens mostly swim downstream. As retention of the increased level of gonadotropins in the organism in case of surfagon injection is only several hours (*Reference book Vidal…*, 2004), the aforementioned effect seems to be caused by hormonal imprinting—a postponed hormonal response of the organism.

The aim of the present study is estimation of the kind and time of surfagon injection on rheoreaction of brown trout *Salmo trutta*.

MATERIALS AND METHODS

The experiment was carried out on juvenile brown trout aged 10–15 months in the aquarial system of the Institute of Ecology and Evolution, Russian Academy of Sciences. The fish were kept in 2.5 × 0.5-m basins of the installation with closed water supply, with a water level of approximately 0.5 m, and an average stocking density of 320 specimens/m^3. The water temperature was 13–15°C, flow rate in basins was 1 cm/s, and illumination was 3000–7000 lx. The fish were fed twice a day with artificial feed Forel Start 55/13 (AQUAREX, RF) granules size 1.2–1.4 mm. The weight of fish at the age of 10 months was 16–18 g, while that at the age of 15 months was 38–42 g.

Prior to injections, the experimental fish were kept for 1–2 min in the solution of anesthetic lidocaine (100–150 mg/L of water) until the motor activity decreased. Surfagon in sodium chloride solution was injected to the experimental fish at the age of 10 months with an insulin syringe intramuscularly under the pectoral fin. To increase the hormonal effect, the injection was done twice (Chebanov et al., 2004) with an interval of 48 h. At the first injection, the surfagon dose was 10 μg/kg of fish, and that at the second injection was 15 μg/kg. The preparation was applied in a dose efficient for rainbow trout (Pavlov D. et al., 2016; Pavlov et al., 2018a, 2018b). The experimental specimens at the age of 10 months were transferred by 80 specimens to two tanks of the size 1.2 × 0.5 m, at the water level of approximately 0.5 m. The control (intact) specimens were transferred into the same tanks in the same quantity. The previous special experiment demonstrated that the response of the fish to manipulation stress related to the injection is not recorded already at the seventh day after the surfagon injection. Therefore, it was not necessary to use an additional control group of fish injected with physiological solution (placebo) for comparison at the selected longer observation periods.

Two series of experiments were performed. **The first series of experiments** (one experimental and one control group) was started 2 days after surfagon injection and the ratio of rheoreaction types were determined during 2 months: the experimental juveniles at the interval of 24 h and the control juveniles at the interval of 48 h. The experiments are made on the fish at the common satiation state. The first series of experiments was aimed at assessment of the kind and time of manifestation of rheoreaction changes in brown trout after surfagon application.

The second series of experiments was aimed at assessments of the longer effect of the surfagon in rheoreaction of brown trout. Two other fish groups were used (experimental and control) kept prior to the experiments under the aforementioned conditions during 5 months (15-month-old fish). The ratio of rheoreaction types was determined in the experimental and control fish in a usual satiation state, while at the second, fifth, seventh, and tenth day of starvation in starving fish. Starvation stimulates individual migratory activity (Pavlov et al., 2010a, 2019b) and may contribute to elucidation of concealed effects of surfagon injection to rheoreaction.

The rheoreaction type was determined in the following installation “fishway”: 2-m long, 0.6-m wide tray separated into nine sections, with the length of transverse walls 0.48 m and 7-cm width of passage between the sections. The water flow is made by a 0.5-kW submersible pump. The flow rate in passages between sections was 20–30 cm/s.

The fish (by 10 specimens) were placed into the start section with water flow, which was closed at both sides with lattices. After 20 min acclimation of fish to the experimental conditions, the lattices of the start section were opened and the number of fish in each section of the “fishway” was recorded in 20 min. The specimens that ascended upstream in sections 1–4 were considered positive rheoreaction type (PRT), those that remained in the fifth (start) section were compensatory rheoreaction type (CRT), and those displaced to downstream sections 6–9 were negative rheoreaction type (NRT) (Pavlov et al., 2010a, 2010b). Eight experiments with the fish of experimental or control groups were made every day. For each experiment made during the day new fish were used. In calculations, the number of specimens in compared groups in a particular section of the installation in all experiments was summed up. The contranatance index (I_k) was calculated by the results of distribution of fish in the “fishway”. This index indicates to which direction in relation to water flow the fish displaced on an average. It changes from 1 (all fish ascended from the start section to the upper section no. 1) up to –1 (all fish descended from the start section to the lower section no. 9). The index was calculated by the following equation: $I_c = \Sigma(n_i(N_s - i))/(N_s - 1)\Sigma n_i$, where n_i is the number of fish in the ith section of the installation, specimens and N_s and i are the number of the start section and of the ith section of the installation.

In statistical processing of the material, Student’s t-test, Student’s test for fractions and ANOVA were used.

RESULTS

The first effect of surfagon injection. At the age of 10 months, the juvenile trout were at the parr stage. Their color was gray with olive shade, with expressed round dark spots all over the body. Both prior to the experiment and in the experimental installation, the behavior of experimental and control fish was individual, and no shoaling reactions were noted. After removal of protecting lattices, some specimens moved to upstream sections, while others moved to downstream sections or remained in the start section.

Two-way analysis of variance demonstrated that the experimental and control fish differed significantly by distribution in sections of the “fishway” ($p < 0.05$) on the 25th–29th day after surfagon injection. The differences between groups were retained up to the 39–43 days after injection ($p < 0.001$ and $p < 0.01$) and were absent from the 46th–50th days.

Differences of ratios of the rheoreaction types between the experimental and control groups of fish were recorded only on the 25th day after injection. In the experimental group, the share of fish significantly increased to 26.4% ($p < 0.05$ by Student’s test for fractions) with PRT; the increase of such fish occurred only at the 39th–43rd day after injection.

The contranatance index in the fish of the experimental and control groups is different by the data of the two-way analysis of variance ($p < 0.001$). It depends on the time passed from the injection time ($p < 0.001$). In the experimental specimens, I_c (–0.45) was higher on average than that in the control ones (–0.56) (Fig. 1). For greater clearness, the change of differences of I_c is shown in specimens of the experimental and control groups in Fig. 2. The surfagon

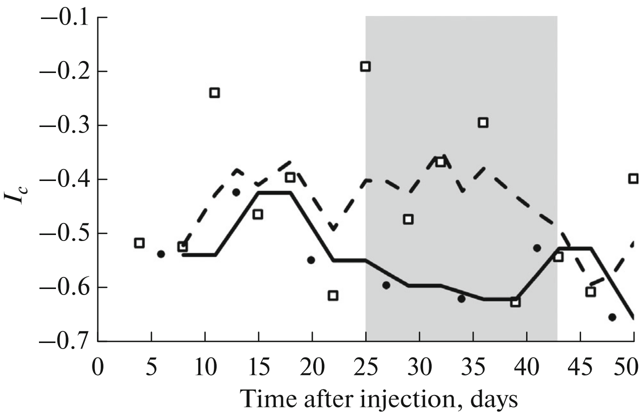

Fig. 1. Dynamics of the contranatance index (I_c) in the experimental (– – –) and control (—) juveniles of brown trout *Salmo trutta* during 2 months after surfagon injection; leveled by four points in the experiment (□) and in the control (●); (■) period during which the reliable differences between the experiment and the control are recorded.

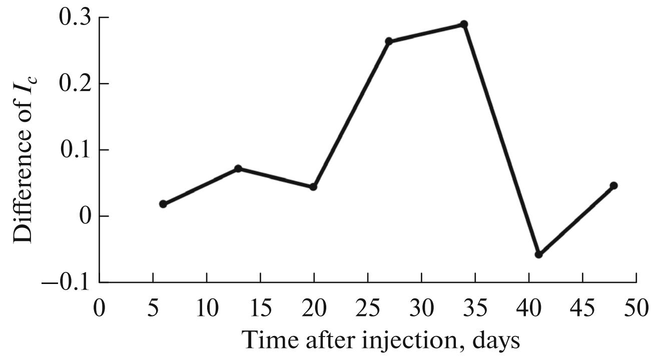

Fig. 2. Dynamics of difference of the contranatance indices (I_c) of the experimental and the control groups of brown trout *Salmo trutta* during 2 months after surfagon injection.

effect on rheoreaction was maximally expressed at the 27th–34th day after injection.

The long-term effect of surfagon injection. Smoltification started in juvenile brown trout up to the age of 15 months both in the experimental and control groups. This was indicated by silvering of fish, which was present to some extent in all fish. Behavior of fish was still individual; no shoaling reactions were observed.

In a common state of satiation, the experimental and control fish did not show significant reactions in rheoreaction (Fig. 3). In both groups, the specimens moved predominantly downstream. In case of starvation, the ratio of rheoreaction types of brown trout changes. The two-way analysis of variance of the change of contranatance index demonstrated that its values depend on the fact whether the fish were injected with surfagon (the experiment or the control) ($p < 0.001$). In the experimental specimens in the process of starvation, this index hardly changed (Fig. 3). In the control fish, it suffered considerable changes during starvation.

DISCUSSION

The fish behavior in the water flow is very variable. Their upstream and downstream migrations or compensatory movements at certain river stretches repeatedly change each other during their whole life. The hormonal system of the organism regulates its physiological state and takes an immediate part in this process. One of regulating hormones of fish behavior in the water stream is GnRH and its analogs. It is known (Plate et al., 2003) that it intensifies the reaction of spawners of sockeye salmon *Oncorhynchus nerka* during the spawning migration stimulating them to overcome the water flow and aquatic obstacles (waterfalls and streamflow thresholds). We previously demonstrated (Pavlov D. et al., 2016) that surfagon, analog of GnRH, also intensifies the upstream swimming in rainbow trout at the age of 2 months.

In the present study, the new materials on surfagon's effect on rheoreaction of another species, *Salmo trutta*, in experiments of a different duration (during 2 months after injection and during 2 weeks in 5 months after injection). The surfagon effect on rheoreaction of juvenile trout is manifested at the 25th day after injection, it is maximally expressed at the 27th–34th day, and is not recorded any more at the 43rd day. The kind of influence is similar to that previously recorded in rainbow trout (Pavlov D. et al., 2016) being intensification of PRT in fish. A similar effect would probably be caused by surfagon on other representatives of the fam. Salmonidae. Taking into consideration that the increased level of gonadotropins in the

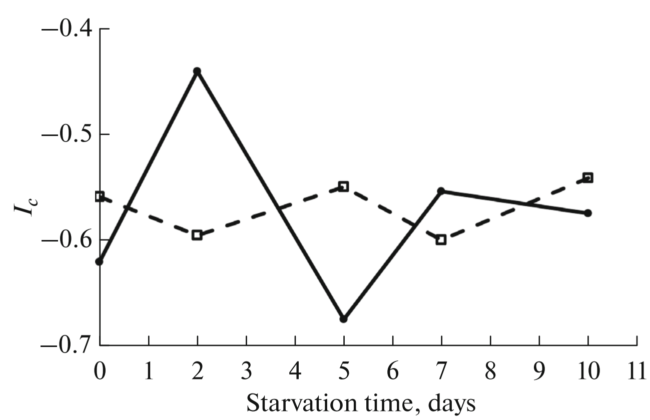

Fig. 3. Dynamics of difference of the contranatance indices (I_c) of the experimental and the control groups of juvenile brown trout *Salmo trutta* at the age of 15 months (5 months after surfagon injection) in common satiation state (0th day) and after starvation (second to tenth day); designations as in Fig. 1.

organism is retained during several hours after application of the preparation it may be assumed that surfagon is a signal factor leading to restructuring of the hormonal regulation of the organism.

The experimental and control group of brown trout at the age of 15 months in a common state of satiation do not differ in the ratio of rheoreaction types. However, it is known that the rheoreaction of fish (the ratio of rheoreaction types) may change considerably in the extremal state under unfavorable life conditions. For example in the case of starvation, the share of fish with PRT decreases generally and share of fish with NRT decreases (Pavlov et al., 2007, 2010). Therefore, it was decided to reveal the hidden surfagon effects by experimental starvation. In case of starvation of the 15-month-old fish, we found differences in behavior of the experimental and control fish. In the experimental fish during the whole starvation period, the contranatance index hardly changed. On the contrary, it demonstrated considerable fluctuations in the control specimens. That is, the intact fish demonstrated the natural behavioral response to shortage of food sources motivating them to search for favorable life conditions (Pavlov D. et al., 2010, 2016; Pavlov E. et al., 2016). In the experimental specimens, surfagon intensifies their adaptation capacities and stabilizes their behavior in the water flow. Thus, the surfagon effect is a long-term one: it is manifested at the experimental starvation of fish after 5 months of injection. It may be supposed that the preparation intensifies adaptive capacities of juveniles to the available life conditions, which decreases their motivation for migration—changes the trajectory of fish development towards residence.

The principal function of GnRH and of its analogs is directed to gonadal development. For example, surfagon injection accelerates maturation of gonads in rainbow trout (Pavlov et al., 2018a, 2018b). It was repeatedly reported (Thorpe, 1994; Pavlov and Savvaitova, 2008; Olsson and Greenberg, 2011; Gruzdeva et al., 2013, 2017; Pavlov et al., 2014) that the resident trajectory of development in salmonids is related to accelerated maturation of their gonads. It is probable that surfagon via intensification of the gametogenesis influences on the hormonal regulation of the organism and modifies rheoreaction and motivational component of fish. This mechanism should be confirmed by additional investigations.

CONCLUSIONS

(1) The first effect of surfagon injection on the rheoreaction of 10-day-old juvenile brown trout is manifested at the 25th day after injection and is recorded during 19 days. This effect is seen as the increase of the share of fishes with PRT: in their swimming predominantly upstream.

(2) A long-term effect on the 15-month-old brown trout rheoreaction is found 5 months after injection. It is manifested only at starvation and is expressed in stabilization of their behavior in the water flow.

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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.

REFERENCES

Boula, D.V., Bernatchez, C.L., and Audet, C., Physiological, endocrine, and genetic bases of anadromy in the brook charr, *Salvelinus fontinalis*, of the Laval River (Québec, Canada), *Environ. Biol. Fish*., 2002, vol. 64, pp. 229–242.

Chebanov, M.S., Galich, E.V., and Chmyr', Yu.N., *Rukovodstvo po razvedeniyu i vyrashchivaniyu osetrovykh ryb* (Manual for Breeding and Growing of Sturgeon Fishes), Moscow: Rosinformagrotekh, 2004.

Chen, C.C. and Fernald, R.D., Distributions of two gonadotropin-releasing hormone receptor types in a cichlid fish suggest functional specialization, *Comp. Neurol*., 2006, vol. 495, pp. 314–323.

Chen, C.C. and Fernald, R.D., GnRH and GnRH receptors: distribution, function and evolution, *J. Fish Biol*., 2008, vol. 73, pp. 1099–1120.

Gruzdeva, M.A., Malyutina, A.M., Kuzishchin, K.V., et al., Regularities of the life history strategy adoption in masu salmon *Oncorhynchus masou* from the Kol River (Western Kamchatka) in regard to the processes of growth and sexual maturation, *J. Ichthyol*., 2013, vol. 53, no. 8, pp. 585–599.

Gruzdeva, M.A., Kusishchin, K.V., Pavlov, E.D., et al., Morphophysiological patterns of life history strategy adoption in Dolly Varden *Salvelinus malma* in Kamchatka, *J. Ichthyol*., 2017, vol. 57, no. 5, pp. 688–705.

Jonsson, B. and Jonsson, N., *Ecology of Atlantic Salmon and Brown Trout: Habitat as a Template for Life Histories,* Fish Fisheries Series vol. 33, New York: Springer-Verlag, 2011.

Ogawa, S., Akiyama, G., Kato, S., et al., Immunoneutralization of gonadotropin-releasing hormone type-III suppresses male reproductive behavior of cichlids, *Neurosci. Lett*., 2006, vol. 403, pp. 201–205.

Olsson, I.C. and Greenberg, L.A., Life history tactics in cohorts of a partial migratory brown trout (*Salmo trutta* L.) population, *ISRN Ecol*., 2011, vol. 2011, art. ID 915239. doi 10.5402/2011/915239

Pavlov, D.S. and Savvaitova, K.A., On the problem of ratio of anadromy and residence in salmonids (Salmonidae), *J. Ichthyol*., 2008, vol. 48, no. 9, pp. 778–791.

Pavlov, D.S., Lupandin, A.I., and Kostin, V.V., *Mekhanizmy pokatnoi migratsii molodi rechnykh ryb* (Mechanisms of Downstream Migration of Juveniles of River Fishes), Moscow: Nauka, 2007.

Pavlov, D.S., Kostin, V.V., and Ponomareva, V.Yu., Behavioral differentiation of underyearlings of the Black Sea salmon *Salmo trutta labrax*: rheoreaction in the year preceding smoltification, *J. Ichthyol.*, 2010a, vol. 50, no. 3, pp. 270–280.

Pavlov, D.S., Kostin, V.V., Zvezdin, A.O., et al., On methods of determination of the rheoreaction type in fish, *J. Ichthyol.*, 2010b, vol. 50, no. 11, pp. 977–984.

Pavlov, D.S., Pavlov, E.D., Ganzha, E.V., et al., Cytological status of the gonads and the level of thyroid and sex hormones in juvenile Black Sea trout, *Salmo trutta labrax*, of two phenotypic forms, *J. Ichthyol.*, 2014, vol. 54, no. 7, pp. 476–484.

Pavlov, D.S., Pavlov, E.D., Kostin, V.V., and Ganzha, E.V., Influence of surfagon on rheoreaction of juvenile rainbow trout, *Russ. J. Dev. Biol.*, 2016, vol. 47, no. 2, pp. 93–98.

Pavlov, E.D., Ganzha, E.V., Ponomareva, V.Yu., et al., Effect of methyltestosterone on physiological state and rheoreaction of rainbow trout *Parasalmo mykiss* (=*Oncorhynchus mykiss*) under unfavorable keeping conditions, *J. Ichthyol.*, 2016, vol. 56, no. 6, pp. 904–915.

Pavlov, E.D., Bush, A.G., and Pavlov, D.S., Estimation of surfagon influence on the gonadal state of rainbow trout *Parasalmo mykiss* (=*Oncorhynchus mykiss*) juveniles at the background of temperature stress, *J. Ichthyol.*, 2018a, vol. 58, no. 3, pp. 417–424.

Pavlov, E.D., Ganzha, E.V., Vo, T.H., et al., Condition of sex glands in the young of the current year of rainbow trout subjected to surfagon injections, *Russ. J. Dev. Biol.*, 2018, vol. 49, no. 2, pp. 108–116.

Plate, E.M., Wood, C.C., and Hawryshyn, C.W., GnRH affects activity and jumping frequency in adult sockeye salmon, *Oncorhynchus nerka, Fish Physiol. Biochem.*, 2003, vol. 28, pp. 245–248.

Soma, K.K., Francis, R.C., Wingfield, J.C., et al., Androgen regulation of hypothalamic neurons containing gonadotropin-releasing hormone in a cichlid fish: integration with social cues, *Horm. Behav.*, 1996, vol. 30, pp. 216–226.

Thorpe, J.E., Reproductive strategies in Atlantic salmon *Salmo salar* L., *Aquacult. Res.*, 1994, vol. 25, pp. 77–87.

Vidal 2003/2004. Spravochnik Vidal'. Lekarstvennye sredstva veterinarnogo nazmacheniya v Rossii (Vidal Handbook 2003/2004: Veterinary Drugs in Russia), Moscow: AstraFarmServis, 2004.

Volkoff, H. and Peter, R.E., Actions of two forms of gonadotropin releasing hormone and a GnRH antagonist on spawning behavior of the goldfish *Carassius auratus, Gen. Comp. Endocrinol.*, 1999, vol. 116, pp. 347–355.

White, S.A., Nguyen, T., and Fernald, R.D., Social regulation of gonadotropin-releasing hormone, *J. Exp. Biol.*, 2002, vol. 205, pp. 2567–2581.

Yamamoto, N., Oka, Y., and Kawashima, S., Lesions of gonadotropin-releasing hormone-immunoreactive terminal nerve cells: effects on the reproductive behavior of male dwarf gouramis, *Neuroendocrinology*, 1997, vol. 65, pp. 403–412.

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