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Original scientific paper

HISTOLOGICAL CHARACTERISTIC FEATURES OF THE RAINBOW TROUT *ONCORHYNCHUS MYKISS* OVARIES (WALBAUM, 1792) GROWN IN VARIOUS MICROAMBIENT CONDITIONS^{1*}

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Abstract: Aquaculture as an agricultural activity in our country is capable of producing large amounts of different types and categories of fish. Climate and geographic characteristics of our country, the terrain and development of agricultural production as well as compliance with the principle of “sustainable development”, the position and importance of fishery in a multipurpose water utilization, must determine the direction of development of the future trout production. The most important type of fish grown in the trout fisheries in BiH is the rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792). In addition to the fact that growing this type of fish is attractive to many producers, considering the high yield potential, it is also characterized by a significant level of tolerance to varying microambient conditions of the environment. Given the importance of fish production in Bosnia and Herzegovina, which is one of the rare branches of food sector that has fulfilled the international standards and criteria and secured access to the markets of the EU countries, it is necessary to establish optimal conditions to ensure high productivity of salmonid fisheries. Studying the reproductive cycles can provide very precise and significant results of the overall condition of the entire organism during this very complex physiological process and of the overall population in the respective ecosystem.

Key words: rainbow trout (*Oncorhynchus mykiss*), gonads, microclimate conditions

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INTRODUCTION

It is known that fishery in our region has a long and successful tradition for almost a century which has been confirmed by a number of scientific and expert papers of eminent experts from this area who occupy a significant place in this field in the world and also by successfully implemented projects of local and global character, freshwater and marine fish farming and processing plants for fish and fish products. Also a large number of endemic fish species and inexhaustible natural resources of high quality river, lake and sea water have to be mentioned. Proper selection of native material in the intensive Rainbow trout farming ensures high production parameters of offspring (Korjenić, 2010). When it comes to intensive production of California trout (*Oncorhynchus mykiss*) it is necessary to satisfy a number of factors that should provide the most favorable conditions for living and rapid growth of the fish population, or contribute to the faster realization of the planned production and marketing of the final product for the market. Above all producers must ensure the quality of the genetic potential of fish, quality foods by age categories, health veterinary supervision and

physico-chemical parameters of water quality, of which the following are the most important ones: sufficient amount of water supplying the fish farm, water temperature, concentration of dissolved oxygen in water and pH value of water (Aganović, 1979; Treer i sar, 2001; Ćuk i sar, 2006; Katavić, 2009). The insignificant changes in the conditions of keeping and nutrition disturb the exploitation of the biological potential of the fish, and over certain limits it endangers the physiological functions which can lead to a disorder of the health condition of the organism (Jeremić et al., 2004). Among the most important external factors in the control of reproduction are: temperature and water quality, dissolved oxygen content, light regime, photoperiod, presence of specific propagation substrate, water flow, tide, annual cycles, nutrition, and possible human intervention through hormone application to stimulate spawning (Sofradžija, 2009; Suljević, 2012). Our research attempted to find out the impact of different micro-ambient conditions on the intensity of oogenesis and the histological image of the Rainbow trout ovary.

MATERIAL AND METHODS OF WORK

The research of the histological characteristics of the Rainbow trout gonads from the three Bosnian fish hatcheries belonging to different basins included field work and laboratory analysis. The material for our research was sampled

from three fish farms in Bosnia and Herzegovina: 1. Royal-fish located on Jablanica Lake (Lower Jablanica) the artificial reservoir on Neretva River 2. Magazine MAPRIM located on stream of Ramić brook (Pazarić-Resnik), which flows

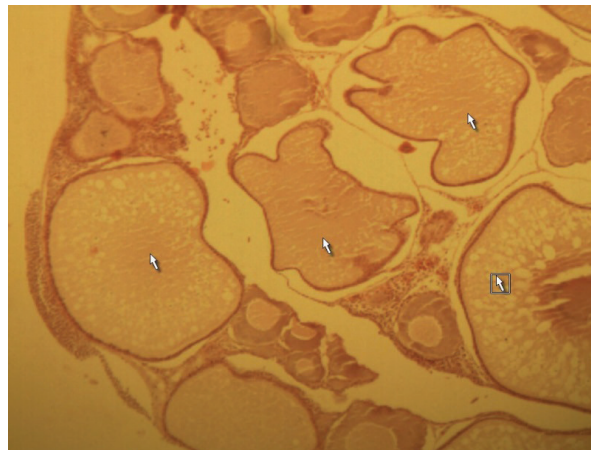
into the Bosna River and 3.Eco Project located on the river Krupica (Jeleć-Miljevina), which, along with Govz, flows into Bistrica and then Drina. During the research, water temperature (° C), altitude, pH value and oxygen saturation were taken into account. For histological study of the ovaries, 24 sexually mature individuals from two to five years were sampled. The gonads were separated into labeled plastic bottles and fixed with 10% formaldehyde and delivered to a laboratory where their further analyses and preparation of histological samples were conducted. The pre-molding process implied dehydration of the samples by passing them through alcohols with increasing concentrations. Samples were first placed in 70% alcohol for two days, then transferred to 96% alcohol for one day and finally to 100% alcohol also for one day. After this procedure, the samples were transferred to a mixture of 100% alcohol and toluene for two hours and then only in toluene for another four hours. Samples prepared in that way were left in paraffin for five hours and then again at twelve hours. With this the process of embedding into paraffin blocks was completed. The method of processing samples from fixation to embedding into paraffin was made on the

rotary tissue processor of the MICROM model STP-120. After finishing the molding process, the samples were cut with a digital microtome of LEICA RM 2145 in several serial sections of 0.5 to 1.5 micrometers. A total of 400 preparations of 80 samples (40 left and 40 right female sex glands) were made. Sections were and placed on slide glasses and deparaffinized by passing them slowly through a series of decreasing concentrations of alcohols. After that sections were painted with hematoxylin and eosin combination, covered with a coverslip, and mounted with Canada balsam. Microscopic preparations were made and analyzed in the Laboratory for Histology and Embryology of Veterinary Faculty in Sarajevo from January 2013 to July 2014. The descriptions of the histological preparations were performed under a light microscope equipped with a MOTIC TYPE 102M marking camera under 100, 200 and 400x magnification. Analyses of the histological structure were carried out using a special Motic Images Plus 2.0 ML software. Microscopic research included the analysis of female gonads during the spawn period by following developmental stages and the degree of ripening at various fish farms.

RESULTS

On Eco-project fish farm located at an altitude of 760 m, the water temperature during the sampling was 7 ° C. The water pH value was 7.8, and the oxygen solubility in water was 10.5 mg / l. On Magazin MAPRIM farm, located at an altitude of 653 m, water temperature was 11 ° C, a pH of 8.3, and a solubility of oxygen 10 mg / l. The measured water temperature on Royal-fish farm, located at an altitude of 202 m, was 8 ° C. The pH of the water was 8.15, and the solubility of the oxygen was 8.27 mg / l.

In the examined histological samples of the ovaries from the Royal fish farm follicles after ovulation were noticeable in a considerably smaller extent than in the ovaries of fish from other fish farms. Postovulatory follicles are characterized by the presence of granulosa, lutein cells, degraded follicles and blood capillaries with free erythrocytes, oval appearance and clearly visible basophilic sails (Figure 1).



Picture 1. Vitellogenesis: white arrows indicate secondary and tertiary developmental stages of oocyte (HE X 200)

Histological preparations of ovaries of individuals raised at the Eco-project (Jeleć-Miljevina location) farm have attracted special attention in the research. Histological studies of samples collected from this farm showed a difference in the histological finding, which was generally observed in the general picture of the ovary (Figure 2).

The parenchyma was abundant with oocytes in the phase of prematuration, maturation and a multitude of postovulatory follicles. After detailed histological analysis, we observed a lot of vitellogenic oocytes of extremely large dimensions, filled with egg yolk granules and fatty vacuoles (Fig. 3). On the surface of these follicles there was a clearly preserved zona radiata.

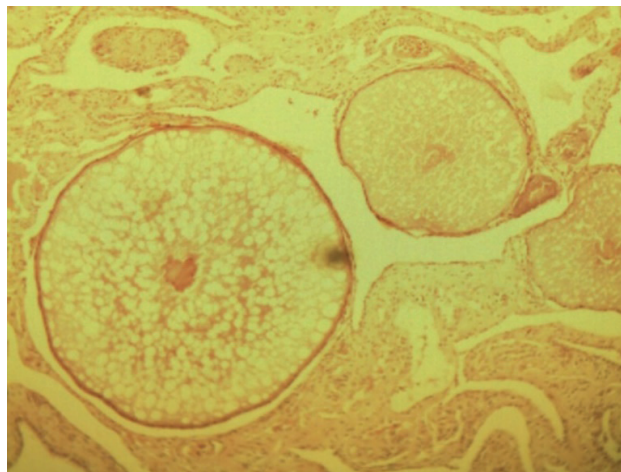


Figure 2. The general picture of the ovary during the spawning period : a multitude of large vitellogenic oocytes (HE X 200)

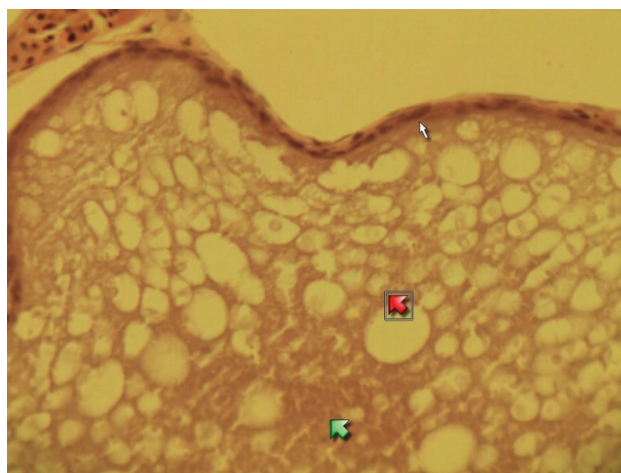


Figure 3. Secondary yolk oocyte: White arrow indicates zona radiata ; green arrow indicates yolk granules; red arrow indicates vacuoles (HE X 400)

Ambient conditions were probably suitable for such intensity of oogenesis. We can conclude that the oogenesis of the fish from this farm in comparison with the other two fish farms, in the same period of the year in November, was at its peak due to the significant

presence of mature oocytes. Fish from the fish farm Eco-project (Jeleć-Miljevina locality) were grown in spring water concrete pools.

Although histological analyzes the Californian trout ovaries were taken

at different locations, showed certain differences in material and the presence of developmental forms of the oocyte, we can say that the ovaries of individuals sampled from the MAGAZIN MAPRIM

farm (Ramici potok-Pazarić location) showed differences in the histological image of the ovary. Right and left gonads were abundant with immature oocytes (Figure 4).

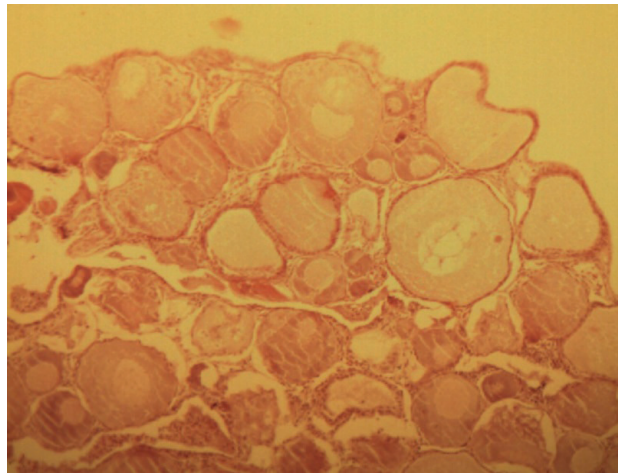


Figure 4. General picture of ovaries during the spawning period of individuals sampled on Magazine MAPRIM farm (HE X 200)

Fish in MAPRIM fish farm are grown in concrete ponds.

DISCUSSION

In the literature, we can find that the photoperiod and other ecological factors strongly influence the reproduction cycle of the Californian trout, and that their manipulation can dictate the spawn period. (Randall, 2001). Variation of ecological factors significantly affects the growth and time interval of gonadal development in fish (Ekanem et al, 2013). Morphological descriptions and detailed descriptions of California trout ovary were presented in their study (Grier et al 2007). Photoperiod and temperature have different effects on different types

of fish. The effect of different water temperatures on the Californian trout results in a different time of spawn and egg size (Pornsoping and Sar, 2007), which explains the results of our histological description of the ovaries. Although the results of the measurements showed different physico-chemical parameters of the water on all three BiH farms, literature sources confirm that they were in the physiological limits required by the Californian trout in intensive farming (Cuk i sar, 2006; Vranić i sar, 2011; Marković & Mitrović-Tutundžić,

2003; Markovic et al., 2006) which is in accordance with our histological description of ovaries. In Californian trout breeding technology it is necessary to provide elementary conditions: pure water with fulfilled oxygen requirements (9-11 mg / l), appropriate temperature

(8-12 ° C), pH value (6.5-8.5) sufficient water flow and systematic nutrition with different types of industrial and natural food (Cuk i sar, 2006, Marković and sar, 2006, Vranić and sar, 2011); fish farms that were the subject of our research basically meet these conditions.

CONCLUSION

The histological analysis of the californian trout ovary samples at various fish farms with different ambient conditions revealed that the highest intensity of oogenesis was in the samples

from the fish farm Eko-projekt, Jeleć-Miljevina locality (pond system with continuously flowing water, altitude 760 m, water temperature 7 ° C, pH 7.8, solubility of oxygen 10.5 mg / l).

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