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Impact of environment parameters and the freshness of the ova on the reproductive performance of *Oncorhynchus mykiss* in Morocco

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Abstract

The objective of the study was to assess the impact of water oxygen concentration and ambient light on the success rate of egg development until hatched rainbow trout (*Oncorhynchus Mykiss*, Walbaum, 1792) as well as the success rate of artificial fertilization according to the freshness of the ova (*in vitro* aging) of the spawners of the Ras Al Ma salmon farming station. The results obtained show that the mean success rate varies between 73% and 69% of the eggs development depending on the oxygen concentration, 76% for the eggs incubated in the dark versus 47% only for the eggs exposed to ambient light.

With regard to fertilization success rates according to their freshness (t0, t1, t2, t3 and t4 hours), the results showed that it is about 71% for the fertilized eggs just after being collected from spawners and gradually decreases to reach only 30% four hours after the stripping.

Keywords: rainbow trout, fertilization rate, egg development, oxygen, light

1. Introduction

Morocco, a country of great hydrographic diversity, has experienced several introductions of fish species, including Rainbow Trout (*Oncorhynchus mykiss*, Walbaum, 1792) [2]. This indigenous salmonid of the west coast of North America is easy to reproduce due to its great adaptability and its tolerance to higher water temperatures compared to the species of the same family [6]. In Morocco, such as in the rest of the world, rainbow trout is mainly used to meet the increased demand for recreational fishing in cold waters and for human consumption [2]. Unlike its original biotope, rainbow trout breeds in Morocco artificially by the National Center for Hydrobiology and Fish farming (CNHP), under the auspices of the High Commission for Water and Forests and the Fight against Desertification (HCEFLCD), production of rainbow trout fry at center level varies according to years and is impacted by environmental conditions. In 2013 production was maximal and exceeded 2, 3 fingerlings [8, 4]. Almost all of this production was used for the restocking of rivers and water bodies of sport fishing whose water conditions were favorable. The objective of this study was to evaluate the productive performance of this fish, depending on some environmental conditions such as the oxygen and light levels imposed during egg incubation period at the CNHP farming station on the one hand, and the fertilization rate of the eggs according to their freshness on the other.

2. Materials and Methods

2.1 Site study

The Azrou fish farming station is located at 33 °26'20.6 " N 05 ° 13'31.5 "W and occurs at the altitude of 1 250 m in the province of Ifrane in the center of the Middle Atlas in Morocco. It is a cold water fish farming unit that belongs to the National Center of Hydrobiology and Aquaculture of Azrou (CNHP). The water supply is provided by a local spring whose temperature is around 14 ° C [1]. Created at the beginning of the 19th century (1924), the station practiced since the artificial breeding of indigenous trout (*Salmo trutta macrostigma*, Dumeril, 1858). Artificial reproduction of rainbow trout began just after its introduction in Morocco in 1925 [10] for recreational purposes, and also with the aim of increasing Moroccan ichthyologic biodiversity [2]. Until 1957, Salmomid farming was practiced only at the Azrou fish station, but with the increase in the number of fishers and the creation of artificial water bodies, production has become insufficient to meet needs and increased demand of this

species. For example, the Salmonid farming station of Ras El Ma was established in 1957, which allowed the increase in rainbow trout production to over 2,000,000 during the 2011-2012 periods [4].

2.2 Effect of oxygen concentration and light during the incubation period

Oxygen is one of the most important parameters during the various phases of the life cycle of fishes [3, 11, 2]. In order to determine the success rate variation during the incubation period as a function of the oxygen concentration, measurements of the dissolved oxygen level per incubator are made 3 times per week using oxygen Orion-Type Model 3301 during the entire incubation period at the Azrou fish farm.

To demonstrate the effect of light during the incubation period, an experiment was developed at the incubation area at Azrou station with two troughs, each containing four incubators with the same number of eggs. The eggs of the first trough were covered by a lid allowing a dark incubation area, whereas the eggs of the second trough were exposed to the ambient light of the incubation area. It is worth noting that the waters flowing in the two troughs have the same origin.

In order to determine the effect of oxygen concentration and the impact of light during the incubation phase, we counted the number of dead or aborted eggs (white eggs) in different troughs.

2.3 Effect of *in vitro* aging of rainbow trout ova on production rate

In order to determine the effect of ova aging on fertilization success rates as well as incubation and hatching, two experiments were carried out on consignments of eggs after extraction from three spawning females.

In first handling, the collected ova were weighed with a balance of 0.1 gram precision and then divided into 5 consignments of the same weight. The first control consignment was fertilized immediately with freshly recovered milt from three males; the fertilized eggs were incubated in the hatchery at the Azrou salmonid farming station. The rest of the ova were stored in the shade at room temperature for fertilization and incubation at regular intervals (1, 2, 3 and 4 hours).

The same principle was maintained in the second manipulation, which consists in following the impact of embryonic development on the aging of the ova, but with different time intervals (1, 2, 3 and 4 days after the stripping) after ova storing at a temperature of 6 ° C.

3. Results and Discussion

3.1 Variation of fertilization as a function of oxygen

The mean values of dissolved oxygen concentration differ from one incubator to another. It was 8.92 mg /l as the maximum value in incubator 1 and 7.75 mg /l as the minimum value for incubator 4 during the incubation period for the 2013/2014 season. The average number of eggs not aborted during this incubation period as a function of oxygen concentration varied from one incubator to another (Fig. 1).

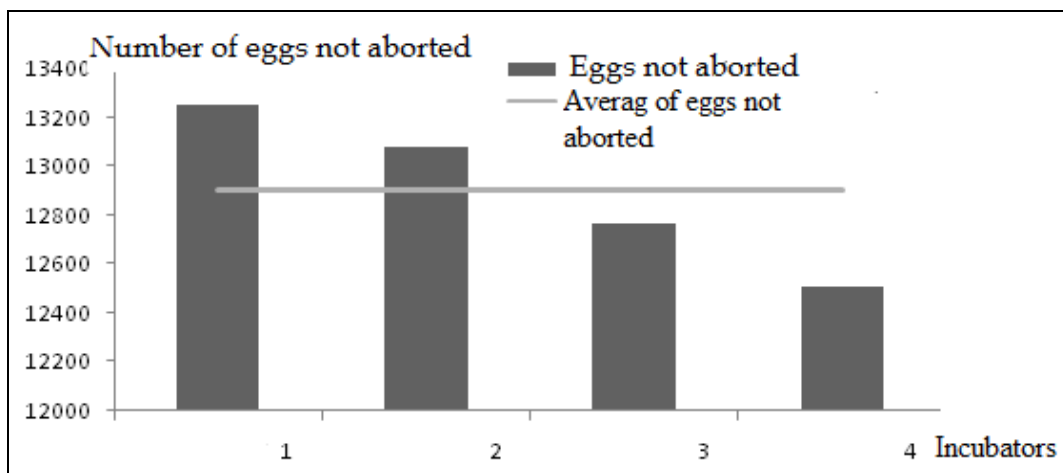


Fig 1: Variation in the number of non-aborted eggs in broodstock of the Ras El Ma salmon farming station according to incubators

The number of not aborted eggs in incubators 1 and 2 was 13250 and 13081 eggs compared to 12901 and 12509 in incubators 3 and 4. The success rate was higher at the level of Incubator 1 (73%) whose oxygen concentration was of the order of 8.92 mg/L, this success rate decreased respectively as a function of the oxygen level and reached 69% in the incubator 4 whose incubator oxygen concentration was 7.75mg /l. Because the temperature and pH of the water were constant throughout the year at the Azrou station (between 13.5 ° C and 14 ° C; pH = 7) [1, 5], and that the eggs were incubated in the dark; these variations can, therefore, only be explained by the difference in oxygen concentration at the level of the different incubators.

The analysis of the one-criterion variance did not reveal any significant difference between the mean of the non-abortive eggs per incubator at the probability threshold of 0.05. There was no significant difference either between the means of success rate, according to incubators at the 5% significance level. This showed that oxygen requirements were satisfactory in the incubation room.

3.2 Impact of light during the incubation period

The effect of light was of paramount importance during egg incubation, hatching and blistering stages in salmonids [9]. The figure 2 presented the results obtained during the incubation period as a function of the exposure or not to the light.

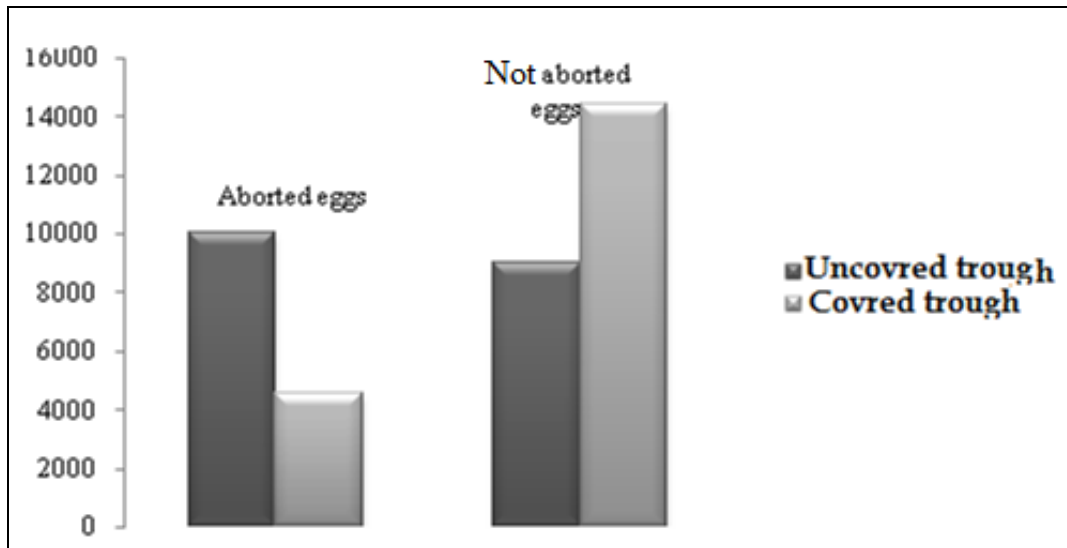


Fig 2: Change in the number of aborted and non-aborted eggs of Rainbow trout spawners in the salmonid aquaculture station as a function of light

The number of aborted eggs in the trough exposed to ambient light was about 10015 eggs, compared to only 4571 white (aborted) eggs in the trough whose eggs were protected from light darkness). These results showed a success rate of the incubation phase ranged from 47% for light incubated eggs to 76% for dark incubated eggs (Results confirmed by the Student test at the significance level 5%). Death of eggs during the incubation period is explained by the fact that the exposure of eggs to light promoted the development of fungi and leads to a higher mortality rate. In addition to this excessive death of eggs by fungal attack that spread rapidly from dead eggs to healthy eggs during this critical phase of embryonic development or manipulations of eggs are discouraged before the eye stage ^[11], the effect of light was a constant stress for embryos and fish fry, leading to a more rapid outbreak, higher mortality rates and smaller fry than

those kept in the dark ^[6].

3-3 Impact of *in vitro* aging of rainbow trout ova

Depending on the freshness of the eggs, the results of fertilization rates vary on average between 71% for fertilized ova just after stripping and 30.75% for fertilization success for eggs kept *in vitro* 4 hours after ovulation. For moderately fresh eggs (2 hours after ovulation), the success rate of fertilization is 50%. This rate decreased very remarkably 24 hours after ovulation. This decline in fecundability was due to the self-activation of the chorion which occurs just after egg extraction. This self-activation of the chorion leads to the closure of the Micropyle that prevents penetration of the sperm ^[12] and consequently decreased the fertilization success rate.

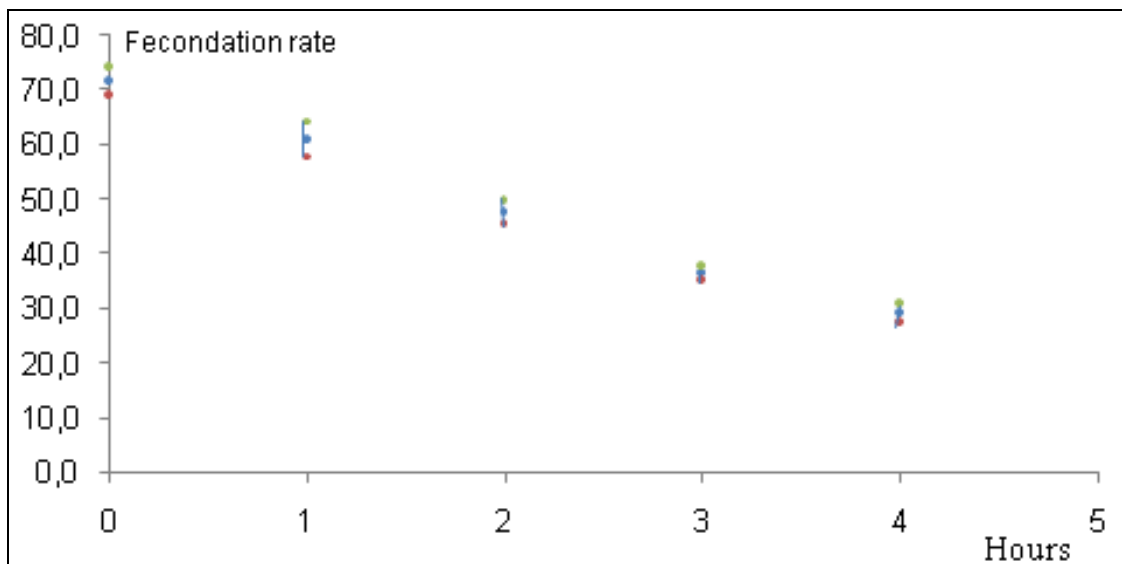


Fig 3: Evolution of the fertilization rate as a function of the aging time of the ova

For the embryonic development of fertilized eggs during the incubation period as a function of aging ova (1, 2, 3 and 4j), the results obtained (Fig. 4) clearly showed that the success rate (embryonic development) was very important at 0 days (yellow color of the eggs) and decreased considerably for the

fertilized eggs one day after ovulation (white color of the eggs = death of the eggs), these results corroborate and confirm the importance of the freshness of the eggs for the other stages following fertilization.

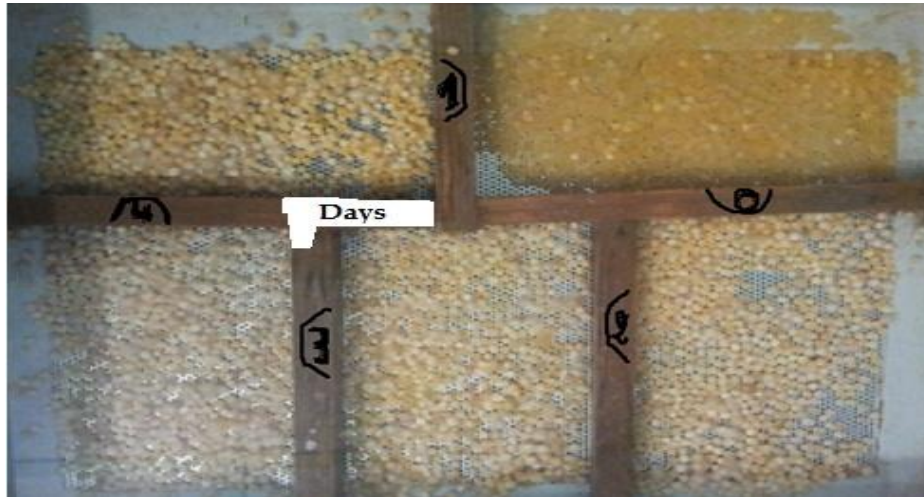


Fig 4: Results of the incubation of rainbow trout Eggs showing the effect of aging *in vitro* in days on the embryonic stage (O = for fresh ovule, 1 = day after ovulation.)

Conclusion

Fish management requires control of the environmental and biological factors of the raised fish species. The following parameters, medium oxygen, freshness of eggs and light level during the incubation phase are decisive in fish production.

Conclusion: Fish management requires the mastery of the first stages of production cycle start-up, namely breeding, incubation and rearing. These different stages make it possible to generate the fish needed for fish production. To achieve its objectives, the farmer must master the different operations while taking into account the environmental factors and biological parameters of the species in question. Among these key factors, one can cite the oxygen of the medium, the freshness of the ova and the level of light during the incubation phase.

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