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Effect of Aging Silver Carp (*Hypophthalmichthys molitrix*) Ova *In Vitro* on Offspring Viability Rates (Beni Mellal Hatchery, Morocco)

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ABSTRACT

The quality of the silver carp (*Hypophthalmichthys molitrix*) eggs is important during artificial reproduction. This quality influences the tolerance of eggs to survive *in vitro* after ovulation and before fertilization. This tolerance of eggs to storage conditions has significant effect on the viability rates of the offspring (fertilization rate, embryo survival rate, larval survival rate and fry survival rate). It is most often recommended that eggs be collected and fertilized immediately after ovulation during seed production. In practice, however, several females injected with pituitary extract hormones will ovulate at the same time, so the collection of eggs of some females must be postponed. The handling of each female takes about 15 to 20 minutes, and the interval between the fertilization of the eggs of the first and the last female is usually more than 60 minutes. During this time, eggs gradually age. The aim of this study was to quantify the maximum *in vitro* residence time of eggs after which they remain suitable for fertilization and to give acceptable progeny viability rates. The study was carried out at the Beni Mellal hatchery in Morocco using 20 females weighing between 2.9 and 9.4kg. Their eggs were fertilized at different time intervals after ovulation (0.30, 60, 90 and 120min). The results obtained show that the viability rates of the offspring are acceptable up to the limit of 45min at 18°C. In order to avoid this aging phenomenon, it is advisable to work in small groups with a reduced number of females and to shift the time intervals between hormone injections.

INTRODUCTION

In Morocco, large dam reservoirs all experience varying degrees of eutrophication, resulting from significant enrichment with fertilizing elements. These elements also promote the excessive development of aquatic plants at the level of irrigation canals by slowing down the flow and distribution of water **(Abouzaid *et al.*, 1986; Cherifi *et al.*,**

2002; Alaoui Mhamdi *et al.*, 2003). This, in turn, has led to the introduction of the silver carp (*Hypophthalmichtys molitrix*) in these bodies.

The initial import occurred in 1983 originating from Hungary followed by a subsequent import in 1987 from Bulgaria. It was from this point that the species began to play a highly significant role in the country. A substantial body of research has demonstrated the species biological and economic efficacy **(Statova *et al.*, 1986; Srivastava & Brown, 1991; Bromage *et al.*, 1992; Brooks *et al.*, 1997)**. However, it is notable that natural reproduction of this species is only observed within the thermal and hydrological conditions characteristic of its native distribution area, specifically within the middle course of Chinese rivers **(Pivnicka & Cerny, 1987)**. In order to address the escalating demands of managerial entities responsible for the provision of drinking water and irrigation canals, while concurrently circumventing the necessity for costly imports of this species from foreign countries, a concerted initiative has been undertaken. Morocco established an artificial reproduction core at the Deroua station in Beni Mellal in 1990. This station is regerded as a paradigm of research and development policy. The findings and observations derived since its creation have propelled our country to the forefront in this domain. It is within this context that the present study is undertaken, with the objective of further optimizing the technique for producing silver carp seeds. The present study aimed to investigate the impact of *in-vitro* aging of the eggs on the viability of the offspring encompassing the fertilization rate, embryo survival rate, larval survival rate and fry survival rate with the objective of quantifying the maximum residence time of the eggs. The eggs will be examined to establish whether they remain fertile and produce offspring with acceptable viability rates. The paucity of literature on this subject is a key point of concern. It has been demonstrated that the prolonged stay of eggs *in vitro* after ovulation induces significant biochemical changes in the eggs and thus affects the different viability rates of the offspring **(Sakai *et al.*, 1975; Hirose *et al.*, 1979; Statova *et al.*, 1986; Navas *et al.*, 1997)**. Consequently, the aging of ovulated oocytes is a pivotal factor to consider when egg laying is induced by hormonal treatment. It is imperative for the fish farmer to be able to determine the precise time of ovulation and to have a clear understanding of the time available for the harvesting and fertilization of the oocytes in order to achieve optimal rates of offspring viability **(Suzuki, 1980)**.

MATERIALS AND METHODS

1. Place of study

The Deroua station is located in the national forest 20km southwest of the city of Béni Mellal in Morocco. It is located in a semi-arid region with mild winters (Latitude= 32° 20’ North, Longitude= 6° 45’ West, Altitude= 428m). It is based on mid-Plioquaternary clay-sand formations **(Emberger, 1930; Bouhachim *et al.*, 2023)**.

2. Infrastructure and material

The nursery ponds and the hatchery are modern with equipment carefully designed and installed while seeking the best reproductive and technical performance than those of other hatcheries which greatly affect the cost of producing carpillons (Fig. 1).

Fig. 1. View of the ponds and the hatchery at Deroua station

3. Source of water supply

The hatchery is supplied with water from a well (Beni Moussa water table). Groundwater is highly recommended in hatcheries **(Stickeny, 1979)**. They have the advantage of being clear and free of solid elements and aquatic predators harmful to the development of eggs, larvae and fry. The ponds are fed with water from irrigation channels coming from the Bine El-Ouidane Dam to optimize yields and exploit all the ecological niches in the water column.

4. Experimental protocol

To study the effect of the aging silver carp eggs outside the ovarian cavity (*in-vitro*) on offspring viability (fertilization rate, embryo survival rate, larval and fry survival rate), 20 females of 2.9 to 9.4kg live weight were artificially reproduced. At the time of ovulation (April and May), the mass of the eggs of each female was collected in a 5-liter container and weighed using a 0.1g precision balance. A batch of 100 to 150g of eggs were fertilized immediately after ovulation, the rest were divided into two groups of four batches of the same weight then covered with a towel soaked in water and stored separately at two different temperatures (ambient 18°C, and 9°C in the refrigerator). The eggs were fertilized at a regular intervals (30, 60, 90 and 120min after ovulation). Each batch was incubated in a 30 liter conical incubator at 22 to 24°C. The 25ml pipette was immersed in the incubator and the eggs and the embryos rise into the pipette by capillary action. The pipette passes through the entire water column in the incubator, providing a homogeneous and representative sample of the batch of incubated eggs. To improve reproducibility, three samples were taken from each incubator to determine each viability rate.

5. Determining of offspring rates

The fertilization rate (FR) was determined 12 hours after fertilization. At this stage, fertilized and unfertilized eggs were easily recognisable under a binocular microscope **(Majdoubi *et al.*, 2017)**.

FR = (Number of fertilized eggs / Total number of eggs) x 100

The embryonic survival rate (ESR) was determined 24 hours after fertilization. At this stage, viable embryos can be identified by their black eyes and somites, whereas aborted embryos have an elongated white mass on the yolk which has begun to disintegrate.

ESR = (Number of viable embryos / Total number of embryos) x 100

Larval survival rate (LSR) was determined 36 hours after fertilization. At the embryonic stage, the samples were separated into boxes of a certain number. The embryos pierced the shell with very energetic movements. Live larvae swam upwards and dead larvae were inactive.

LSR = (Number of live larvae / Total number of larvae at 36h stage) x 100

The fry survival rate (FSR) was determined 56 hours after fertilization. Still in the same boxes as before, the viable larvae remained mobile and devolved to the fry stage and vice versa for the dead larvae.

FSR = (Number of viable fry / Total initial number of larvae) x 100

RESULTS AND DISCUSSION

1. Effect of *in-vitro* egg aging on fertilization rate and embryo survival rate

Figs. (2 and 3) show the results of egg fertilization and embryo survival rates (FR and ESR) obtained after ovulation and fertilization of silver carp eggs at different *in vitro* aging times (0, 30, 60, 90 and 120min) at two storage temperatures (ambient 18°C and in the fridge 9°C).

At T0, the comparison between the different FR and ESR obtained for each female shows a very great intrinsic deference. This could be due to a difference in egg quality. Good quality eggs give better fertilization and embryo survival rates than poor quality eggs **(Craik, 1985; Billard *et al.*, 1986)**. In this study the average FR and ESR varied between 67.63 (±3.4) and 56.3% (±5.6) respectively. These results are not significantly different (P=0.05) from those obtained by other authors **(Mc Evoy, 1984; Springate *et al.*, 1984; Majdoubi *et al.*, 2017)**.

At different *in vitro* aging times (30, 60, 90 and 120min) and at different storage temperatures (18 and 9°C), it was observed that the FR and ESR remained low throughout the prolonged *in vitro* ageing of the eggs. This could be explained by the biochemical changes that the eggs undergo under the influence of the storage environment. Poor egg storage conditions lead to the deterioration of egg quality. **Craik**

(1985) and **Billard *et al.* (1986)** suggest that variability in egg quality is due to hereditary and non-hereditary factors. Non-hereditary factors are usually environmental and nutritional factors.

The FR and ESR show a very large variability for the two storage temperatures (18 and 9°C). They decrease significantly at 9°C. Despite the simultaneous fertilization of the eggs, the highest rates were recorded at a tempreature of 18°C. These results show that the storage temperature of the eggs has a significant effect on the survival rate of the offspring. They also show that eggs are better preserved at room temperature (18°C) than at refrigerator temperature (9°C).

In conclusion, egg quality is a factor that greaty influences TF and TFE. In the hatchery we recommend working at room temperature (18°C) and fertilizing the eggs immediately after ovulation to minimize the effect of prolonged storage on egg quality.

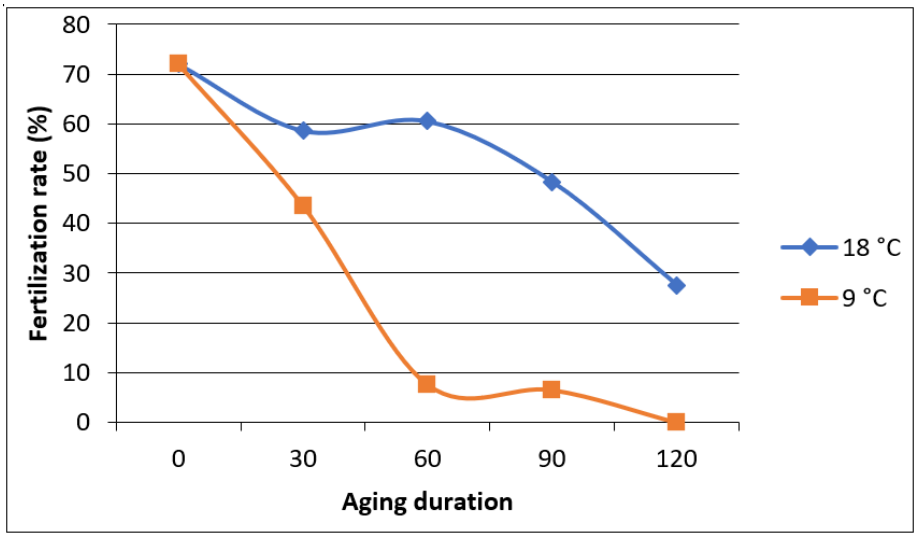

Fig. 2. Evolution of fertilization rate means after *in vitro* aging of eggs in minutes

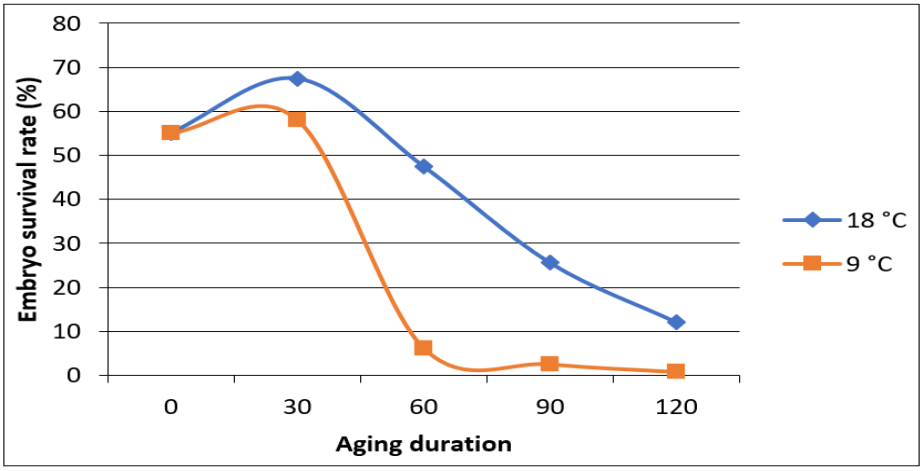

Fig. 3. Evolution of the means of embryo survival rates after *in vitro* aging of the eggs in minutes

2. Effect of *in-vitro* egg aging on larval and fry survival rates (LSR and FSR)

Figs. (4, 5) present the results of the different larval and fry survival rate (LSR and FSR) obtained in this study at different time intervals of *in-vitro* egg aging (0, 30, 60, 90 and 120min) at two different storage temperatures (ambient 18°C and refrigerator 9°C).

The LSR and FSR oscillate, respectively, between a maximum of 100 and 51.81% and a minimum of 6.22 and 00% at 18°C, and between a maximum of 69.44 and 33.66% and a minimum of 00 and 00% at 9°C. These results show a very high variability as in the case of egg fertilization and embryo survival rates. This could be explained by the multiple effects of modifiable and non-modifiable factors on egg quality **(Hirose *et al.*, 1977; Cerda *et al.*, 1995; Evans *et al.*, 1996)**. Modifiable factors are those related to the effects of prolonged *in-vitro* aging and storage temperature. Non-modifiable factors are those generally associated with hereditary factors **(Craik, 1985; Billard *et al.*, 1986)**. In addition to intrinsic factors (egg quality), TSL and TSA can be influenced by hygienic conditions in aquaria and nursery tanks **(Folch *et al.*, 1957; Cerda *et al.*, 1994)**. Poor hygienic conditions can result in total mortality of larvae or juveniles.

The multiple correlation between the different rates of progeny viability (FR, ESR, LSR and FSR) showed that high FR and ESR do not always imply good LSR and FSR. This multiple correlation also showed that progeny viability rates are high and acceptable up to the limit of a duration estimated at 45min at 18°C ambient storage temperature. In the case of 9°C, this duration does not exceed 20min. Beyond that, in both cases, the viability rates of the progeny are unacceptable and the operations become laborious and very expensive.

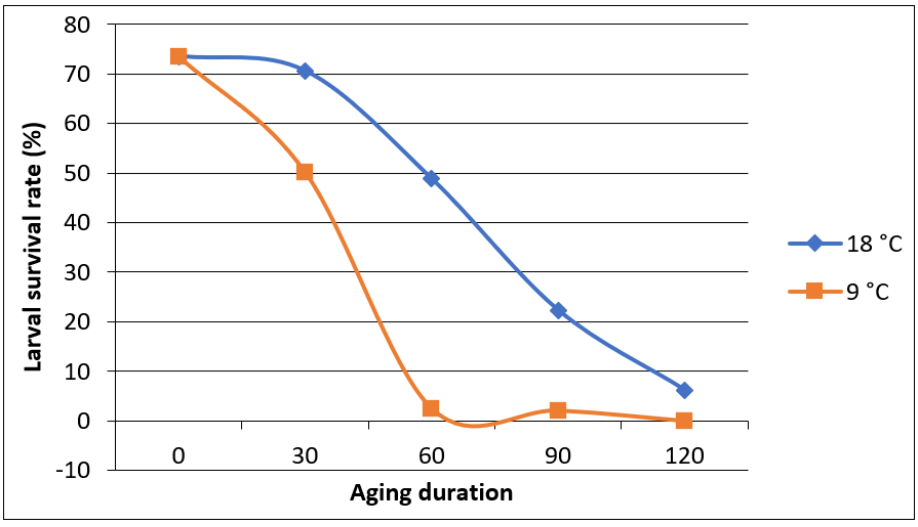

Fig. 4. Evolution of the means of larval survival rates after *in vitro* aging of the eggs in minutes

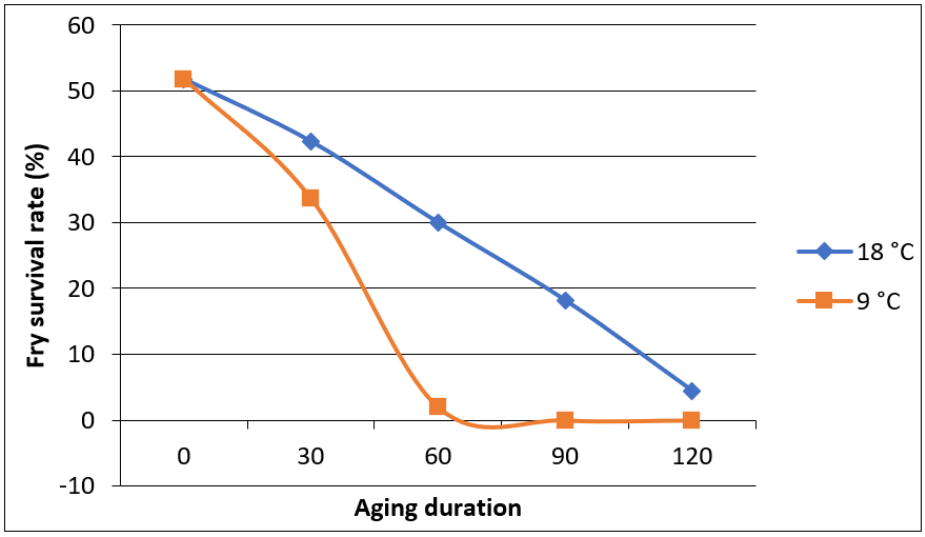

Fig. 5. Evolution of the means of fry survival rates after in vitro aging of the eggs in minutes

CONCLUSION

This study quantified the maximum duration for *in vitro* storage of the silver carp ova while maintaining acceptable offspring viability rates (fertilization rate FR, embryo survival rate ESR, larval survival rate LSR, and fry survival rate FSR) at two temperatures: ambient (18°C) and refrigerated (9°C). The results obtained show that the storage of eggs *in vitro* at 18°C room temperature is more favorable than at 9°C refrigerator temperature. At 18°C, fry survival rates remain acceptable for up to 45 min unlike at 9°C where they don't exceed 20min. Beyond that, in both cases, the eggs become unsuitable for fertilization and give very low rates of offspring viability. As a result, the operations become laborious and very expensive. In conclusion, in order to optimize the productive capacity of the silver carp in seed production, it is advisable to fertilize the eggs immediately after ovulation within a maximum of 45 minutes and to work in good hygienic conditions at the ambient temperature of the hatchery. This is done by programming hormone injections to small groups of females which allows fish farmers to reduce the ageing time of the eggs. Small groups of females allow fish farmers to reduce the aging time of the eggs. In addition, the care of the broodstock and the technical skills and experience of the staff play a crucial role in the success and improvement of these operations.

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