



Survival of Landlocked Fall Chinook Salmon Eggs after Delayed Fertilization

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Abstract: Delayed fertilization can be a tool to improve salmonid spawning efficiencies. This study subjected eggs from landlocked fall Chinook salmon (*Oncorhynchus tshawytscha*) to one of three treatments: fertilization immediately after egg removal from female brood stock, fertilization delayed for two hours post-egg removal, or fertilization delayed for four hours. There were no significant differences in egg survival to the eyed-stage or fry hatch between the immediate fertilization and two-hour delay treatments. However, survival to the eyed-stage and survival to fry hatch were significantly reduced in the eggs fertilized after a four-hour delay. These results indicate that landlocked fall Chinook Salmon eggs can be successfully stored for up to two hours prior to fertilization with no negative effects on egg survival.

Keywords: Chinook salmon, *Oncorhynchus tshawytscha*, delayed fertilization, spawning, eggs.

1. INTRODUCTION

During artificial spawning, salmonid eggs are typically fertilized immediately after removal from female broodstock [1]. However, fertilization can be delayed to accommodate egg shipment, facilitate labor use during spawning, or to improve spawning efficiencies [2-5].

Short-term storage of salmonid eggs is generally considered safe. Dahlberg et al. [6] found no impact on sockeye salmon (*Oncorhynchus nerka*) egg survival when fertilization was delayed for 20 minutes, although, Leitritz and Lewis [1] suggest a 24-hour limit on unfertilized salmonid egg storage to maintain maximum survival. Fertilization has been successfully delayed for 8 to 20 hours for pink salmon (*O. gorbuscha*) [7,8], 12 to 96 hours for sockeye salmon [8,9], and 36 to 124 hours for chum salmon (*O. keta*) [8,10,11] depending on storage temperatures. Much longer time frames of up to 24 days for storing salmonid eggs at very low temperatures have been also been reported [5, 12]. However, fall Chinook salmon (*O. tshawytscha*) eggs may be particularly sensitive to storage time and temperature, with 47% mortality observed from eggs stored at 1°C for 48 hours[8]. Similarly, substantial mortality of landlocked fall Chinook salmon eggs stored for only four hours post-stripping has also been reported [13].

Landlocked fall Chinook salmon eggs from Lake Oahe, South Dakota are typically fertilized immediately after stripping and then allowed to water-harden for at least one hour prior to four-hour shipment to a hatchery for incubation. While the water-hardening process should prevent any post-spawning mortality associated with egg shipment [14], survival of Lake Oahe salmon eggs is still considerably less than that observed in eggs from Chinook salmon in their native range [15,16].

In an initial study nearly 20 years ago, Barnes et al. [13] obtained 15.5% survival in Lake Oahe salmon eggs to the eyed-stage when fertilization was delayed by four hours, compared to 31.4 % survival from eggs immediately fertilized. Given the age of this study, and the changes occurring in the Lake Oahe Chinook salmon population over the intervening years [16], further experimentation was needed to re-examine the possibility of delayed fertilization. Thus, the objective of this study was to determine the potential effects on egg survival of delaying fertilization of landlocked fall Chinook salmon eggs for up to four hours after stripping from female broodstock.

2. METHODS

Landlocked fall Chinook Salmon from Lake Oahe were spawned at Whitlocks Spawning Station, near Gettysburg, South Dakota on November 1, 2017. Milt was collected from four males, pooled in a container, and approximately 50 ml placed into an 0.95 L plastic bag. Oxygen was added to the bag prior to sealing and then it was placed on ice until used. Eggs from the eight different females were expressed from each female into separate suspended mesh nets using compressed oxygen [17].

Forty-five eggs were removed from the spawn of each female and divided into three, 0.95 L plastic bags (15 eggs/bag). This was repeated for each of the eight spawns (24 total bags). Three treatments were used: immediate fertilization, two hour delayed fertilization, and four hour delayed fertilization. The bags containing the eggs with the immediate fertilization time were fertilized by adding one ml of milt from the pooled container. Lake water (10° C, total hardness as CaCO₃, 210mg/L; pH, 7.6; total dissolved solids, 390mg/L) was then added to the egg/milt mixture to activate the sperm. The remaining sperm was placed on ice (approximately 1° C) for later use.

After approximately one minute, the eggs were rinsed, poured into another plastic bag with fresh water, placed in a cooler on ice, and allowed to water-harden for one hour prior to incubation. Additional groups were stored at 10° C, fertilized two and four hours later, and processed identically as the immediate-fertilization groups. This process was repeated for the eggs of eight different females, with each group maintained discretely during fertilization, water-hardening, and through incubation. Egg incubation occurred by placing each group of eggs into a discrete 9.5cm plastic petri dish. The egg dishes were incubated in an Insignia model NS-WC16BK6 refrigeration unit at 10°C through complete hatch. Each petri dish contained 30 ml of water, which was changed every seven days until the eyed stage of egg development (incubation day 28) and every three days thereafter, using the technique described by Neumiller et al. [18]. Dead eggs and fry were manually removed and counted when the water was changed. Fry were also removed and counted after hatch. The following formulas were used to determine percent survival: Survival to Eyed Egg Stage (%) = 100 x (number of eyed eggs/initial egg number); Survival to Hatch (%) = 100 x (number of hatched fry/initial egg number).

Data was analyzed by one-way analysis of variance using the SPSS (9.0) statistical analysis program (SPSS, Chicago, Illinois). Significance was predetermined at P < 0.05. Percentage data was log transformed to stabilize the variances [19].

3. RESULTS AND DISCUSSION

Egg survival to both the eyed stage of egg development and to hatch was significantly reduced when fertilization was delayed for four hours (Table 1). However, no significant differences in survival to either developmental stage were observed between those eggs fertilized immediately or at two hours post-fertilization.

Table 1. Mean (\pm SE) survival of landlocked fall Chinook salmon subjected to one of three fertilization times (n=8, means in a column followed by different letters are significantly different).

Fertilization Time	Survival (%)	
	Eyed Stage	Hatch
Immediate	43.8 (14.1) z	41.9 (14.0) z
Two Hour Delay	42.9 (12.8) z	41.0 (13.0) z
Four Hour Delay	31.4 (6.9) y	28.6 (9.7) y

The results of this study support the observations of Barnes et al. [20], who observed decreased survival of landlocked fall Chinook salmon eggs where fertilization was delayed for four hours. The lack of impacts on egg survival by delaying fertilization for two hours indicates that very short-term storage of these eggs is relatively safe, similar to that reported by Dahlberg et al. [6]. However, Lake Oahe landlocked Chinook salmon cannot be stored for the lengthier time periods used successfully by others [5,7, 9-11, 12]. The decreased survival after only four hours of gamete storage observed in this study are likely due to the unique features of the completely landlocked and freshwater Chinook salmon population in Lake Oahe population of Chinook salmon [15, 16, 21]. The 43% egg survival to the eye-up stage for the immediate and two hour delayed fertilization samples in this experiment is greater than that typically experienced with Lake Oahe Chinook salmon eggs [15, 16], and is probably due to selecting likely-viable eggs for inclusion in the experiment.

It is possible that the results of this study could have been influenced by milt storage issues. However, Chinook salmon milt appears to be much less sensitive to storage than eggs [8], and successful salmonid milt storage has been reported for up to 34 days [22]. Bencic et al. [23] successfully stored Chinook salmon milt in a variety of conditions for up to 14 days, and the inclusion of oxygen and gas exchange in the current study should have provided additional benefits [24-27]. Both sockeye and pink salmon milt maintained high fertilization rates when stored at temperatures below 3.2°C [28]. Sperm motility was also likely maintained by the low temperature used for milt [11,29, 30]. Specifically with Lake Oahe salmon, Reese et al. [31] stored milt just on ice in closed containers for up to two hours with loss of motility.

The results of this study indicate that fertilization of landlocked fall Chinook salmon eggs from Lake Oahe could be successfully delayed for up to two hours. Although on a practical level, this time frame would not allow for the four-hour transfer of gametes from the spawning station to the hatcheries (Cleghorn Springs State Fish Hatchery and McNenny State Fish Hatchery, near Rapid City and Spearfish, South Dakota, USA, respectively), it does raise the possibility of gamete shipment to the hatcheries from other locations. Broodstock salmon are frequently electro fished from multiple locations on Lake Oahe, and then transported to Whitlocks Spawning Station [19, 32]. Some of these electro fishing sites are only three hours from a salmon hatchery, opening the possibility of transporting gametes from these electro shocked broodstock directly to a hatchery. However, for this to occur, the females would need to have ovulated. In addition, the fish health issues associated with transfer of unsterile gametes that could not be adequately disinfected directly to a production fish hatchery would also need to be resolved [33-35].

4. CONCLUSIONS

This experiment showed that the fertilization of landlocked fall Chinook salmon eggs could be delayed up to two hours, with egg mortality occurring somewhere between two and four hours post-removal from the female broodfish. Additional research should be conducted to determine optimal egg storage conditions, including the possible use of antibiotics [35], and the relationship between storage temperature and successful egg storage times [5].

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