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Delayed Fertilization of Landlocked Fall Chinook Salmon Eggs Stored with Oxygen at Two Temperatures

Hunter Eide and Michael E Barnes*

McNenny State Fish Hatchery South Dakota Department of Game, Fish and Parks Spearfish, South Dakota State University, United States

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***Corresponding author**

Michael E Barnes, McNenny State Fish Hatchery, South Dakota Department of Game, Fish and Parks Spearfish, South Dakota, United States,
Tel: 605-642-1262;
Email: mike.barnes@state.sd.us

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Abstract

This study examined the use of supplemental oxygen and two temperatures (1° and 11°C) during the four-hour storage of unfertilized landlocked fall Chinook salmon (*Oncorhynchus tshawytscha*) eggs from Lake Oahe, South Dakota, USA. There was a significant and positive effect of oxygen use on egg survival to the eyed-stage and hatch. In addition, survival to egg eye-up and hatch was significantly affected by storage temperature, with decreased survival at 1°C. However, there was no significant interaction observed between the use of oxygen and storage temperature. Mean survival to hatch ranged from 50% for those eggs stored with oxygen at 11°C compared 17.8% for those eggs stored on ice at 1°C in air. To maintain landlocked Chinook salmon egg fertility, storage with supplemental oxygen at 11°C is recommended.

Introduction

While fertilization frequently occurs immediately during artificial spawning of trout and salmon, gametes can also be stored for use at a later time [1]. Delayed fertilization may be done in an attempt to increase egg survival or to improve the efficiencies of spawning operations, particularly if spawning occurs far from incubating hatcheries [2-6].

Successful egg storage prior to fertilization has been shown to be influenced by the amount of time the eggs are stored and the storage temperature [2]. In one of the earliest studies involving salmonids, sockeye salmon (*Oncorhynchus nerka*) unfertilized eggs were successfully stored without any loss of fertility at temperatures of 5°C for 25 hours, but decreased fertility was noted in eggs stored at either 8°C and 11°C [7]. Withler and Morley [8] observed that unfertilized sockeye salmon eggs could be stored at temperatures of either 2.9° C or 9.9°C for up to 22 hours without any loss of fertility. However, after 70 hours of storage, fertility decreased by over 50%, but only during storage at 9.9°C. Similarly, unfertilized pink salmon (*O. gorbuscha*) eggs stored at 8.5°C experienced declining fertility after only eight hours of storage, while those at 3.2°C maintained egg fertility over five times longer (46 hours). Poon and Johnson [2] also found pink salmon eggs remained fertile for up to six hours after removal from female broodstock when stored at 6°C. Jensen and Alderdice [9] reported a negative relationship between egg storage time and temperature with unfertilized chum salmon (*O. keta*) eggs, with egg fertility remaining for up to 124 hours at 3°C storage temperatures, and Ginatullina et al [6], successfully stored unfertilized rainbow trout (*O. mykiss*) eggs at 2°C for 20 hours. 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 [10].

Unlike milt, which is typically stored with the air replaced by gaseous oxygen [11], fish eggs are generally stored in ovarian fluid with air. However, significant increases in fertility have been observed with Mozambique tilapia (*Oreochromis mossambicus*) eggs stored for 20 hours in oxygenated conditions compared to just air [12].

Lake Oahe, South Dakota, USA, contains a relatively unique population of landlocked fall Chinook salmon [13]. There is no natural reproduction in this population; it is maintained entirely artificial propagation [14]. Spawning occurs at Whitlocks Spawning Station adjacent to the lake, and fertilized and water-hardened eggs are then transported four hours to production hatcheries. Two studies have evaluated delayed fertilization of Lake Oahe salmon eggs. Barnes et al [15]. reported over a 50% drop in fertility when Lake Oahe salmon eggs were stored for only four hours. More recently, Eide and Barnes [16] also documented that fertilization was impaired when Lake Oahe salmon egg fertilization was delayed for four hours, but noted that egg fertility was maintained for up to two hours. Neither Barnes et al [15] nor Eide and Barnes [16] evaluated the impact of different storage temperatures or oxygen on Lake Oahe Chinook salmon egg storage. Thus, the objective of this study was to determine if decreased storage temperatures and the addition of oxygen could allow such eggs to maintain fertility for up to four hours.

Table 1: Mean (SE) percent survival to egg eye-up and fry hatch of landlocked Chinook salmon eggs fertilized immediately after spawning (control) or stored for four hours prior to fertilization in bags containing either air or compressed oxygen (N = 12, except for the control where N = 6).

		Oxygen		
	Control	Yes	No	P
Eye (%)	43.3 (4.5)	46.1 (5.3)	26.7 (4.0)	0.003
Hatch (%)	42.2 (4.1)	43.3 (5.9)	26.1 (3.9)	0.004

Materials and Methods

Landlocked fall Chinook salmon from Lake Oahe were spawned at Whitlocks Spawning Station, near Gettysburg, South Dakota, USA on October 10, 2018. Milt was collected from males, pooled in a container, and approximately 50 ml placed into a 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 five female salmon were pneumatically-expressed using compressed oxygen. These eggs were then combined into a common pool, with 450 eggs removed for experimentation. The 450-egg group was further divided into 15-egg samples, with each sample placed into its own discrete 0.95 L plastic bag.

A 2 x 2 experimental design was used. With the exception of six egg bags that were fertilized immediately, all of the egg samples were stored for four hours prior to fertilization. The treatments were either stored at 11˚C or with ice (approximately 1˚C), with the bags either containing air or having the air replaced with compressed oxygen (six bags per treatment; N = 6). Egg fertilization occurred by adding one ml of milt to each bag. Lake water (total hardness as CaCo3, 210mg/L; pH, 7.6; total dissolved solids, 390mg/L) was then added to the egg/milt mixture for sperm activation.

After approximately one minute, the eggs were rinsed, with each bag of eggs placed into a discrete 9.5-cm plastic Petri dish filled with 30 ml of water. The dishes were incubated in an Insignia model NS-WC16BK6 refrigeration unit (Best Buy, Richfield, Minnesota, USA) at 10˚C through complete hatch using the technique described by Neumiller et al [17]. 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 two-way analysis of variance using the SPSS (9.0) statistical analysis program (SPSS, Chicago, Illinois, USA). Significance was predetermined at $P < 0.05$. Percentage data was log transformed to stabilize the variances [18].

Table 2: Mean (SE) percent survival to egg eye-up and fry hatch of landlocked Chinook salmon eggs fertilized immediately after spawning (control) or stored for four hours prior to fertilization at either 1˚C (on ice) or 11˚C (N = 12, except for the control where N = 6).

		Temperature		
	Control	1°C	11°C	P
Eye (%)	43.3 (4.5)	27.2 (4.5)	45.6 (5.0)	0.017
Hatch (%)	42.2 (4.1)	27.2 (4.5)	42.2 (5.8)	0.035

Table 3: Mean (SE) percent survival to egg eye-up and fry hatch of landlocked Chinook salmon eggs stored for four hours prior to fertilization in bags containing either air or compressed oxygen and at one of two temperatures (N = 6).

Oxygen	Temperature	Eye (%)	Hatch (%)
Yes	11°C	55.6 (8.0)	50.0 (10.7)
Yes	1°C	36.7 (4.8)	36.7 (4.8)
No	11°C	35.6 (2.8)	34.4 (2.7)
No	1°C	17.8 (5.6)	17.8 (5.6)

Results

There was a significant and positive effect of oxygen use on survival to the eyed-stage and hatch (Table 1). In addition, survival to egg eye-up and hatch was significantly affected by storage temperature, with decreased survival at 1˚C (Table 2). However, there was no significant interaction observed between the use of oxygen and storage temperature with survival to the eyed-stage ($P = 0.922$) or hatch ($P = 0.804$). Mean survival to hatch ranged from 50% for those eggs stored with oxygen at 11˚C compared 17.8% for those eggs stored on ice at 1˚C in air (Table 3).

Discussion

The results of this study describe for the first time the successful maintenance of landlocked Chinook salmon egg fertility after four hours of storage. Storing the eggs with supplemental oxygen was critical. Barnes et al [15] observed significantly decreased landlocked fall Chinook salmon egg fertility when the eggs were stored in air for four hours at undefined temperatures. Additionally, Eide and Barnes [16] also were unable to maintain landlocked Chinook salmon egg fertility after four hours of storage in air at 11˚C. The results of this study, and those of Barnes et al [15] and Eide and Barnes [16], strongly suggest that using oxygen during egg storage for four hours is essential for successful fertilization. In the only other published study examining the use of oxygen during fish egg storage, Harvey and Kelley [12], also noted improvements in the fertility of tilapia eggs after 20 hours of storage prior to fertilization. In contrast to the landlocked fall Chinook salmon eggs used in this study, storage of other salmonid eggs without supplemental oxygen has generally been successful for durations much longer than four hours [2,6-9].

It is surprising that egg fertility was negatively related to storage temperature in this study. In general, colder temperatures are associated with longer salmonid egg storage times [2]. Numerous studies have documented that at lower temperatures ranging from 2˚C to 5˚C, salmonid egg fertility was unaffected for time frames of 22 to 124 hours, compared to declines in fertility at storage temperatures ranging from 8˚C to 11˚C [6-9]. However, Piper et al. [10] reported nearly 50% mortality in Chinook salmon eggs stored at 1˚C for 48 hours. It is possible that the 1˚C temperature used in this study was too cold, although Ginatullina et al [6], successfully stored unfertilized rainbow trout eggs at 2˚C for 20 hours, and pink and chum salmon eggs have been successfully stored at temperatures close to 3˚C [8,9].

While possible, it is unlikely that milt storage influenced the results of this study. Survival was similar among the control, immediately-fertilized, eggs and the eggs subjected to some of the delayed-fertilization treatments. Salmonid milt in general can be

stored for over a month [19], and Chinook salmon milt in particular has been successfully stored for up to 14 days [20]. Sperm motility has been observed in milt from Lake Oahe Chinook salmon after short-term storage on ice [21]. Indeed, the use of low temperatures [9,22] and supplemental oxygen [8, 23-25] in this study likely preclude any issues with sperm storage.

Although overall egg survival in this study was relatively low, it is normal for landlocked fall Chinook salmon eggs from Lake Oahe [13,26]. This introduced, totally-freshwater, population of Chinook salmon is relatively unique, with reproductive characteristics dramatically different than Chinook salmon in their native range [13,26]. This is likely why Lake Oahe Chinook salmon eggs do not exhibit the same response to storage and delayed fertilization as reported for other populations [1,10,16,26].

The successful fertilization of Lake Oahe salmon eggs after four hours of storage, enables the option of shipping gametes from the spawning station to the incubating hatcheries, as opposed to the current practice of immediate fertilization and subsequent shipment of water-hardened eggs [15]. However, disinfection of stored eggs and milt upon arrival at the hatcheries would need to occur [27]. Unlike the long-established techniques using iodophor solutions to disinfect of water-hardened eggs [28-30], potential protocols for gamete disinfection are currently unknown.

Conclusion

In this study documents for the first time the successful storage of Lake Oahe landlocked fall Chinook salmon eggs for four hours prior to fertilization by adding oxygen and maintaining temperatures at 11°C. Future experiments should determine how long eggs can be stored with supplemental oxygen, as well as the impact of different storage temperatures.

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