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Characteristics of landlocked fall chinook salmon producing either viable or completely non-viable eggs

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Abstract
Landlocked fall Chinook salmon *Oncorhynchus tshawytcha* from Lake Oahe, South Dakota, USA, frequently produce spawns where the eggs become visibly dead within four hours after fertilization. This study compared the characteristics of spawning females that produced dead eggs to those that produced spawns with at least some egg survival. No significant differences occurred between the groups of females in total length, post-spawn weight, the number of days held prior to perceived ovulation (ripeness), or egg size. However, females that produced spawns with no egg survival were held at for approximately one day longer after perceived ovulation compared to females producing spawns with at least some egg survival. In addition, total fecundity was significantly lower in the spawns which produced only dead eggs. Hatchery personnel can use this information to decide if spawns should be retained for further processing.

Keywords: landlocked fall chinook salmon, spawning, eggs, lake oahe, South Dakota

1. Introduction
The landlocked, and completely fresh-water, population of fall Chinook salmon *Oncorhynchus tshawytcha* in Lake Oahe, South Dakota, USA, is relatively unique [1, 2]. It is sustained entirely by artificial spawning and hatchery incubation [3], and typically experiences poor reproductive success [1, 2]. Spawning of feral broodstock occurs each fall at Whitlock Spawning Station, near Gettysburg, South Dakota, USA, and eggs are transported approximately 360 km to hatcheries for incubation [4]. After hatching, the resulting fish are typically reared for five months before being transported and stocked into Lake Oahe.
The poor reproductive success of Lake Oahe salmon is exemplified by the complete mortality of all the eggs of some of the spawns of individual females either immediately after water-hardening or after four-hour transport to a hatchery. These observations of egg mortality are possible because live and dead salmonid eggs can be easily distinguished. In comparison to relatively transparent, yellow or reddish-colored live eggs, dead eggs are opaque and at least partially white in color [5-8].
The reason for the total egg mortality in these individual female spawns is currently unknown. It is possible that the eggs are stripped too long after ovulation [9, 10]. However, because it is impractical and nearly-impossible to determine the timing of ovulation in this feral population of Chinook salmon, the identification of more easily-visible characteristics that might be related to this near-total egg mortality phenomenon is desirable. Thus, the objective of this study was to identify any female reproductive or spawn characteristics that could possibly indicate spawns where all of the eggs eventually die versus those containing viable eggs. The ability to identify non-viable spawns would reduce hatchery labor requirements and costs by eliminating unnecessary husbandry associated with egg inventory and incubation.

2. Materials and Methods
2.1 Spawning
Seventy landlocked Lake Oahe fall Chinook salmon were spawned at Whitlocks Spawning Station near Gettysburg, South Dakota, USA on 25 October and 1 November, 2017. Ripe females were anesthetized in a carbon dioxide solution. Following anesthetization, eggs were

expressed pneumatically using compressed oxygen into a mesh collection net. The eggs from each female were then fertilized in lake water for two minutes with pooled milt from two males. Following fertilization, the eggs were rinsed and allowed to water harden in fresh lake water.

2.2 Egg incubation
After water hardening, eggs were transported approximately four hours to McNenny State Fish Hatchery, Spearfish, South Dakota, USA. Upon arrival, the eggs were disinfected with 100 mg/L buffered free iodine (Western Chemical, Ferndale, Washington, USA) for 10 minutes, inventoried using water displacement [11], and placed into discreet vertical-flow incubation trays (MariSource, Fife, Washington, USA). Fish with spawns resulting in obvious total mortality (all of the eggs were opaque and at least partially white) [2, 3, 4, 5] were inventoried and discarded. Each spawn was maintained discretely during spawning, fertilization, transportation, and, for those spawns with viable eggs, subsequent incubation. Well water (11ºC; total hardness 360 mg/L $CaCO_3$; alkalinity as $CaCO_3$, 210 mg/L; pH 7.6; total dissolved solids 390 mg/L) at 12 L/min was used throughout incubation. Formalin treatments of 1,667 mg/L were applied daily (37% formaldehyde, 6 to 14% methanol; Paracide-F, Syndel, Ferndale, Washington, USA) for 15 minutes [6] using a Masterflex model 7524-00 microprocessor peristaltic pump (Cole-Parmer Instrument Company, Vernon Hills, Illinois, USA).

2.3 Egg survival determination
Any dead eggs from the viable spawns were removed on incubation day 30 (eyed egg stage) and the remaining viable eyed eggs were re-inventoried by water displacement. Survival (%) to eyed stage of development was determined by using the following formula:

Survival (%) = 100 x (number of eyed eggs / initial egg number)

2.4 Data analysis
T-tests were conducted to compare the reproductive characteristics and egg survival between the viable and inviable eggs. All data analysis was done using SPSS (9.0) statistical analysis program (SPSS, Chicago, Illinois, USA), with significance predetermined at $p < 0.05$.

2.5 Ethics
This experiment was carried out within the American Fisheries Society Guidelines for the Use of Fishes in Research and within the guidelines of the Aquatics Section Research Ethics Committee of the South Dakota Department of Game, Fish and Parks.

3. Results
Reproductive characteristics were for the most part similar between the 41 females producing spawns with viable eggs compare to the 29 females that had spawns with complete egg mortality (Table 1). AT 698 and 697 mm, mean total lengths were nearly identical between the groups. Post-spawn weights were also very similar, and differed only by slightly more than 2%, which was not significantly different. There was also no significant difference in egg size between the groups.
Both groups were only held on average for one day prior to perceived ovulation.
However, females which produced spawns where all of the eggs died were held significantly more days after perceived ovulation (mean ± SE, 2.4 ± 0.4) than females where at least some of the eggs survived (1.3 ± 0.3 days). Also, females producing spawns with viable eggs had significantly more eggs (2,628 ± 164) compared to females which had spawns with total egg mortality (2,109 ± 172).

Table 1: Mean (± SE) reproductive characteristics for landlocked fall Chinook salmon females producing spawns with either no viable eggs or some viable eggs. Means in a row with different superscript letters are significantly different (n=70; $p<0.05$).

	Viable Eggs	
Characteristic	**No**	**Yes**
N	29	41
Survival to eye (%)	0.0 ± 0.0	27.54 ± 3.04
Length (mm)	698 ± 15	697 ± 14
Post-spawn weight (g)	3,320 ± 245	3,399 ± 217
Days at station to ripe	1.0 ± 0.6	1.0 ± 0.3
Days held at station past ripe	2.4 ± 0.4^a	1.3 ± 0.3^b
Egg size (mm displaced)	4.08 ± 0.15	4.27 ± 0.16
Fecundity	$2,109 \pm 172^a$	$2,628 \pm 164^b$

4. Discussion
Female Chinook salmon that are held longer in the raceways waiting to be spawned have likely missed their prime ovulation window. In fact, 76% of the Chinook salmon that entered the spawning station in 2017 were already ripe as determined by manual palpation. This implies that their window for producing viable eggs from spawning may be reduced as they could have spent considerable time searching for suitable spawning habitat before arrival at the station or prior to capture by electrofishing. Delaying spawning from females with unknown ovulation days has been shown to decrease the reproductive success of Atlantic salmon (*Salmo salar*) eggs [12]. Likewise, rainbow trout (*Oncorhychus mykiss*) have been shown to have a great degree of egg survival variability when their ovulation date was unknown [9]. Salmonids in general have higher susceptibility to overripening and reduced survival of their eggs compared to other species [10]. While increasing the frequency of spawning Lake Oahe Chinook salmon may allow for eggs to be collected in closer proximity to ovulation, this has not been shown to improve egg survival (Nathan Huysman, unpublished data).
Previous experiments have attempted to find a way to identify non-viable Lake Oahe Chinook salmon eggs. Barnes *et al*. [1] was unable to correlate their initial estimates of egg quality with actual survival. Likewise, Barnes *et al*. [8] stated that subjective evaluations of egg quality are mostly unreliable.

5. Conclusion
The results of this study indicate that if egg availability is not a concern, spawns suspected of future total egg mortality can be discarded if they contain relatively few eggs and the females producing the spawn have been held for a relatively longer duration at the spawning station. However, if egg supplies are limited, suspect spawns should be processed and retained until complete egg mortality is visible.

6. Acknowledgements
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