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Landlocked Fall Chinook Salmon Egg Survival during Jar and Tray Incubation at a Production Hatchery

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Abstract: This study compared the survival of landlocked fall Chinook salmon *Oncorhynchus tshawytscha* eggs incubated in either upwelling jars, either with or without daily formalin treatments, or vertically-stacked trays treated daily with formalin in a production hatchery. In the first year of the study, survival to the eyed-egg stage was significantly greater in eggs incubated in jars without formalin compared to trays, but there was no significant difference in survival to hatch between the treatments. In the second year, there were no significant differences in eggs incubated in trays, in jars without formalin treatments, and in jars with formalin treatments. In the final year, there was no significant difference in eyed-egg survival between eggs incubated in trays and jars without formalin, but survival to hatch was significantly greater in the eggs incubated in jars. Jar incubation is recommended to maximize the survival of landlocked fall Chinook salmon eggs.

Keywords: Chinook salmon, egg, formalin, incubation, jars, *Oncorhynchus tshawytscha*, trays.

INTRODUCTION

Salmonid eggs are incubated at production hatcheries using both vertically-stacked trays and upwelling jars [1-3]. Vertically-stacked trays have the advantage in that they use minimal floor space, require relatively small amounts of water, and allow for the handling of individual trays without disturbing the entire stack [1-3]. However, regular treatments of formalin or hydrogen peroxide must be used to control fungus (water molds) until at least the eyed-egg stage [1, 4], and are recommended for use until hatch during tray incubation of landlocked fall Chinook salmon *Oncorhynchus tshawytscha* eggs [5]. While formalin is the most suitable chemical for fungal control, its use poses serious risks to human health [6, 7], and techniques to minimize or eliminate formalin use in aquaculture are extremely desirable.

Compared to the lack of egg movement in trays, eggs are lightly rolled in incubation jars due to upwelling water [2]. Jars can hold more eggs per unit than trays, but require more space and use relatively more water [1]. Unlike vertically-stacked trays, jars can be inexpensive to make [8-10]. Anti-fungal chemical treatments may not be required, as Rach *et al.* [11] showed by using high flow rates during trout egg incubation in small experimental jars. However, no information is available on the use of high flows with trout and salmon eggs in larger production jars. There is also a paucity of published material examining salmonid egg incubation in trays compared to jars. Thus, the objective of this study was to evaluate the effects on the survival of landlocked fall Chinook salmon eggs incubated in either trays or jars with or without daily formalin treatments.

METHODS

All experimentation occurred at McNenny State Fish Hatchery, Spearfish, South Dakota. Landlocked fall Chinook salmon eggs were spawned as described by Barnes *et al.* [12]. Upon arrival at McNenny the eggs were disinfected in a 100 mg/L buffered iodine solution for 10 min prior to placement in either vertically-stacked trays (MariSource, Fife, Washington) or 15.2 cm diameter upwelling jars (Eagar Inc, Salt Lake, Utah). Manufacturers' recommended capacities are 12,000 trout eggs per 11 L volume tray and 16,000 trout eggs per 7.5 L volume jar. Well water (11°C; 360 mg/L total hardness as $CaCO_3$; 210 mg/L alkalinity as $CaCO_3$; 390 mg/L dissolved solids; 7.6 pH) supplied both incubator types. Flows were set at 12 L/min, which was the minimum required for slight rolling of the eggs in the jars, and are the same flows typically used during landlocked fall Chinook salmon egg incubation in trays [5, 13]. Eggs were inventoried by water displacement [1] prior to placement into the incubation units.

On incubation day 32, dead eggs were removed from each incubation unit (individual jar or tray) and re-inventoried to determine survival to the eyed-egg stage. The remaining viable eggs were then placed back into their respective incubation unit. At complete hatch on incubation day 44, dead eggs and fry were removed and counted from each incubation unit. Percent survival to the eyed stage of development and hatch were determined by the following formulas:

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% eye-up = 100 x (number of eyed eggs / initial number of green eggs)

% hatch = 100 x [1- (total mortality during incubation / initial number of green eggs)]

The study began in 2009 and was replicated with some changes in 2012 and 2013. Methods unique to each study year are as follows:

2009 Methods

Eggs were obtained from spawning operations at Lake Oahe, South Dakota. Three jars and three trays each received 4,700 eggs (1,000 mL at an egg size of 4.7 eggs/mL of water displaced). Eggs in the trays received daily 15-min treatments of 1,667,000 µg/L formalin (Parasite-S® Western Chemical, Ferndale, Washington), via a peristaltic pump (Cole-Parmer, Vernon Hills, Illinois), throughout incubation. Eggs in the jars did not receive formalin treatments.

2012 Methods

Eggs were obtained from spawning operations at Lake Sakakawea, North Dakota. Three incubation trays and six jars each received 8,250 eggs (1,500 mL at 5.5 eggs/mL). Similar to 2009, treatments consisted of three trays receiving daily 1,667,000 µg/L formalin treatments and three jars that did not receive any formalin. An additional treatment whereby three jars received daily 15-min treatments of 1,667,000 µg/L formalin was included in 2012.

2013 Methods

Eggs were obtained from spawning operations at Lake Oahe, South Dakota. Three incubation trays and three jars each received 5,000 eggs (1,000 mL at 5.0 eggs/mL). Treatments consisted of three trays receiving daily 1,667,000 µg/L formalin treatments and three jars that did not receive any formalin.

Percent survival data was arcsine square root transformed [14] prior to statistical analysis (SPSS 9.0). In 2009 and 2013, data was analyzed using Student's t-tests. Data collected in 2012 was analyzed by one-way analysis of variance and Tukey's mean comparison procedure. Significance was predetermined at $P \leq 0.05$.

RESULTS AND DISCUSSION

Egg survival was generally similar among the incubation types, with or without jar formalin treatments (Table **1**). In 2009, egg survival to eye-up was significantly greater ($P = 0.035$) in the jars than the trays, but there was no difference in survival to hatch ($P = 0.13$) despite a relatively large difference in treatment means. However, small sample sizes may have precluded any determination of statistical significance [15].

In 2012, there were no significant differences in either survival to eye-up ($P = 0.81$) or hatch ($P = 0.81$) among the treatments. In 2013, there was no difference in survival to eye-up between the jars and trays, but survival to hatch was significantly greater with jar incubation. However, the hatching results for the eggs in the trays during 2013 should be viewed with caution. During one of the last daily formalin treatments, the automatic timer for the peristaltic pump ran longer than the prescribed 15-min treatment. Although unanticipated longer duration formalin treatments were not directly observed, possible formalin-induced egg mortality may explain the relatively large mortality from eye-up to hatch observed in the tray-incubated eggs in 2013 [16].

Table 1. Mean (SE) percent survival of landlocked fall Chinook salmon eggs either incubated in vertically-stacked trays or upwelling jars, with or without daily 15-min 1,667 mg/L formalin treatments. Means with different letters in a column in a given year are significantly different ($P \leq 0.05$; N = 3).

		Percent Survival	
	Formalin	**Eye-up**	**Hatch**
2009			
Trays	yes	49.5 ± 1.3 a	46.5 ± 1.3
Jars	no	55.0 ± 1.2 b	52.3 ± 1.5
2012			
Trays	yes	23.9 ± 2.2	21.2 ± 1.4
Jars	yes	24.9 ± 0.9	22.9 ± 1.0
Jars	no	25.4 ± 1.4	18.7 ± 1.6
2013			
Trays	yes	45.0 ± 1.4	32.1± 1.3 a
Jars	no	48.3 ± 1.6	44.1± 1.5 b

The results of this study indicate that incubating salmon eggs in jars or trays are both acceptable methods. However, a disadvantage of tray incubation is the need to use chemicals to control fungal growth. Hydrogen peroxide and formalin are both approved in the United States for fungal control on salmonid eggs [17], but formalin is more effective at fungal control and less toxic to developing landlocked fall Chinook salmon eggs [13]. However, formalin has been identified a suspected human carcinogen [6], and has defined exposure limits for hatchery personnel [7]. It is also relatively expensive, particularly when shipping costs are included, and requires hatchery staff time and special equipment to dispense safely.

Although this study indicates that salmon eggs can be incubated in upwelling jars without any antifungal chemical treatments, jars do have the disadvantage of requiring more space and water than vertically-stacked incubation trays [1-3]. Using small experimental jars, Rach *et al.* [11] concluded that the flow needed for fungal control was relative to the size of the jar and the number of eggs. Flows too low led to the proliferation of water molds, while flows too high caused egg mortality due to excessive egg movement [11]. The 12 L/min used in the current study produced enough egg rolling to prevent fungal growth. Based on the lack of mortality in the jars in comparison to the trays, 12 L/min was also evidently not high enough to kill these landlocked fall Chinook salmon eggs.

In 2012, soft egg disease (pre-mature hatch) was observed in all of the incubation units. This disease occurs sporadically with landlocked fall Chinook salmon eggs incubated at McNenny [18] and has been observed in other salmonid hatcheries as well [19, 20]. Barnes *et al.* [18] indicated that formalin treatments provided some remediation against the lethal effects of soft-egg disease. In 2012, approximately 10% of the eyed eggs died from eye-up to hatch in both the jars and trays that received daily formalin treatments. However, over 25% of the eyed eggs died prior to hatch in the jars that were not treated with formalin.

The relatively consistent results from this study occurred over a large range of egg survival. Typical egg survival to the eyed-stage of development is approximately 40% in landlocked fall Chinook salmon from Lake Oahe [12]. Egg survival in this study was nearly 55% in the first year of the study, dropped to 25% in the second year, and rebounded to 45% in the final year.

Although no other studies have been published comparing the use of jar and tray incubators, it is highly probable that the results from this study would apply to the incubation of eggs from other salmonids. Egg incubation in trays is relatively standardized among this group of fish [1-3], and egg anatomy and physiology are also very similar [21, 22].

Provided there are no space or water limitations, landlocked fall Chinook salmon egg incubation in jars without any anti-fungal chemical control is recommended based on the reduction in chemical and labor costs, the elimination of potentially-lethal formalin treatment errors, and the elimination of human health risks due to formalin exposure. However, if an outbreak of soft-egg disease in landlocked fall Chinook salmon is anticipated because of egg flaccidity, daily formalin treatments during jar incubation may be necessary as suggested by Barnes *et al.* [18].

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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