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Thermal Tempering Does Not Increase the Survival of Eyed Salmonid Eggs

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Abstract

Eyed salmonid eggs can be subjected to large temperature swings during development, particularly when shipped in ice-filled containers from broodstock facilities to production hatcheries. This study examined the effect of thermal tempering on the survival of brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss* eyed eggs to hatch. Eyed eggs at 1˚C were either directly placed in 11˚C water or allowed to thermally temper from 1˚C to 11˚C gradually over an hour. In addition, each of the tempering treatments occurred either with or without a 10-minute iodophor disinfection treatment. Tempering had no significant effect on survival-to-hatch in either species (85% - 97%). However, survival was significantly lower in rainbow trout eggs subjected to iodine disinfection (73% - 75%) compared to non-disinfected eggs (91% - 97%), likely because of the close proximity to hatch. The results of this study indicate that slowly raising the temperature (thermal tempering) of brown trout and rainbow trout eyed eggs is unnecessary. Additionally, iodine disinfection of eyed salmonid eggs in close proximity to hatching should be avoided.

Keywords

Tempering, Trout, Eggs, Salmonid, Iodophor

1. Introduction

Large numbers of live salmonid eggs are routinely transported around the world [1]. These eggs are typically shipped at the eyed stage of development in moist air containers [2]. Because ice is used in the shipping containers and shipment frequently occurs during the winter months in the northern latitudes due to spawning times for most salmonids, these eggs commonly arrive at the destination hatchery at temperatures only slightly above freezing. Slowly tempering (adjusting) the eggs to the receiving hatchery water temperature is a common and recommended practice [3] [4] [5]. However, the research to support this practice is severely limited.

Eggs are normally disinfected, after arrival, to reduce the risk of introducing pathogenic microbes [3] [6] [7]. Povidone iodine is the preferred disinfectant for salmonid eggs because of its relative safety, effectiveness, and low toxicity to eggs [6] [8] [9] [10] [11]. The U. S. Food and Drug Administration allows the use of povidone iodine at 100 mg/L active iodine for 10 minutes to surface disinfect salmonid eggs [12].

This study was undertaken because of the lack of published research documenting the need for salmonid egg tempering. In addition, there is no information regarding the potential interaction between tempering and iodine disinfection. Thus, the objective of this study was to evaluate the use of tempering, with and without iodine disinfection, on the survival of brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss* eyed eggs.

2. Methods

This study examined tempering and disinfection using a 2 × 2 factorial design. Four treatments were used: 1) Both tempering and disinfection, 2) Tempering without disinfection, 3) No tempering with disinfection, and 4) Neither tempering nor disinfection (**Table 1**). Tempering occurred by placing eggs into 1˚C water, with temperatures slowly increased over one hour to 11˚C. Disinfection occurred by placing the eggs in a solution of 100-ppm active iodine (Ovadine, Syndel Co., Ferndale, Washington, USA) for 10 minutes.

Eyed eggs were received at McNenny State Fish Hatchery, rural Spearfish, South Dakota after overnight shipment in moist air containers with ice. Arrival temperature of the eggs was 1˚C. Immediately after checking the temperature, 300 eggs were removed from the container, and 15 eggs were placed into 20, 10-cm, Petri dishes. Five dishes were used for each of the four treatments (n = 5). Well-water (total hardness as $CaCO_3$, 360 mg/L; alkalinity as $CaCO_3$, 210 mg/L; pH, 7.6; total dissolved solids, 390 mg/L) was used for all experiments. Incubation temperature was 11˚C.

Table 1. 2 × 2 factorial design of experiment. Eggs that were tempered were gradually (over an hour) brought from 1˚C to 11˚C, if eggs were not tempered, they were placed directly into 11˚C water after arrival. Eggs that were disinfected were immersed in 100 ppm active iodine for 10 minutes.

Treatment	Tempering		Disinfection	
	Yes	No	Yes	No
1	×		×	
2	×			×
3		×	×	
4		×		×

For eggs that were both tempered and disinfected, the Petri dishes contained an iodophor solution at 1˚C. After ten minutes, the eggs were rinsed with 1˚C water and placed back into clean Petri dishes also containing 1˚C water. The dishes were then put into a refrigeration unit (NewAir, Orange County, California, USA) and tempered for one hour to 11˚C. For eggs that were only tempered, the undisinfected eggs were placed into the refrigeration unit and tempered for one hour to 11˚C. Petri dishes with eggs that were only disinfected and not tempered contained the iodophor solution at 11˚C. After ten minutes, the eggs were rinsed with 11˚C water, and placed back into dishes containing 11˚C water prior to incubation. Petri dishes of eggs that were neither tempered nor disinfected contained 11˚C water. All of the dishes were incubated using the technique described previously [13] [14]. Each experiment lasted until all eggs had either hatched or died. Percent survival was calculated (1).

$$\text{Survival}(\%) = 100 \times \text{Number of fry/initial egg number} \quad (1)$$

Two trials were conducted, with one using brown trout eyed eggs and one using rainbow trout eyed eggs. Brown trout (Plymouth strain) eyed eggs were received at McNenny Hatchery on November 25, 2019 from Saratoga National Fish Hatchery (Saratoga, Wyoming, USA). Rainbow trout (Arlee strain) eyed eggs were received on December 2, 2019 from Ennis National Fish Hatchery (Ennis, Montana, USA).

Data were analyzed using the SPSS (24.0) statistical program (IBM, Armonk, New York, USA) using two-way analysis of variance. Significance was predetermined at $P < 0.05$.

3. Results

Brown trout eyed egg survival-to-hatch was not significantly different among any of the treatments (**Figure 1**). There was also no significant interaction between tempering and iodine disinfection. There was no significant effect of tempering on rainbow trout eyed egg survival-to-hatch (**Figure 2**). However, egg survival was significantly less in the eggs that received iodine disinfection. There was no significant interaction between tempering and iodine disinfection.

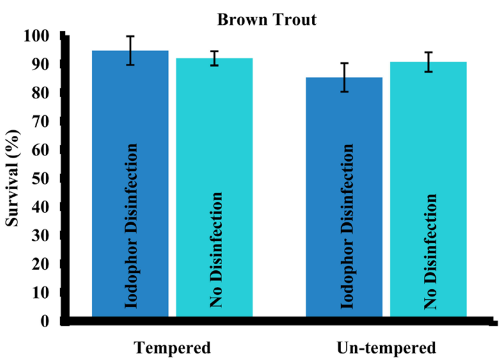

Figure 1. Mean (±SE) percent survival to hatch of brown trout *Salmo trutta* eyed eggs which were tempered or un-tempered, with or without iodophor disinfection.

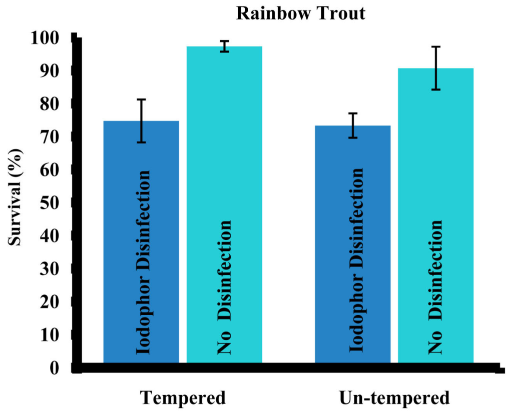

Figure 2. Mean (±SE) percent survival to hatch of rainbow trout *Oncorhynchus mykiss* eyed eggs which were tempered or un-tempered, with or without iodophor disinfection.

4. Discussion

The results of this study indicate that tempering of brown trout and rainbow trout eyed eggs from near freezing during transfer to 11˚C water is not needed. This study contradicts other published recommendations [3] [4] [5] to temper salmonid eggs to water temperatures of the receiving hatchery. Weber *et al.* [15], using rainbow trout eggs prior to the eyed stage, also reported no difference in the survival when subjected to either a rapid or four-hour temperature switch from 5˚C to 10˚C. However, tempering may be needed for salmonid eggs going from cooler to warmer water within six hours of fertilization [16].

This study only exposed brown trout and rainbow trout eyed eggs to temperatures from near freezing to 11˚C, and the 11˚C used in this study is within the optimum range for incubating brown trout and rainbow trout eggs [15] [17] [18] [19] [20]. It is unknown if tempering would be necessary for eyed brown trout and rainbow trout eggs subjected to temperature ranges above 11˚C or those outside their optimal thermal range. It is also unknown if the results from this study would be similar using eyed eggs from other salmonid species.

The increase in mortality of the rainbow trout eggs receiving iodophor disinfection was likely due to the advanced developmental stage of these eggs. Disinfection of salmonid eyed-eggs in a buffered iodophor solution of 100 mg/L active iodine is a safe and well-documented practice [3] [6] [21] [22] [23] [24] [25]. However, hatchery salmonid fry are much more susceptible to iodine toxicity than eggs [25]. Piper *et al.* [3] state that iodophor disinfection treatments should not be used on salmonid eggs within five days of hatching to avoid iodine-induced fry mortality. Avoidance of iodine exposure close to egg hatching is widely recommended [4] [26].

It is very unlikely that the increase in temperature used in this study led to skeletal deformities or other issues identified with heat shock in Atlantic salmon *Salmo salar* [27] and zebrafish *Danio rerio* [28]. These negative effects are caused by multiple, long-period (24-hour) heat shocks at stages of development prior to egg eye-up. The eyed eggs as used in this experiment were at a much later stage of development and were not exposed to high temperatures outside of their optimal incubation range.

In conclusion, this study clearly shows that tempering of brown trout and rainbow trout eyed eggs arriving at 1˚C to at least 11˚C is unnecessary and will not improve egg survival. Further experimentation is needed to determine if tempering is beneficial for more than the 10˚C increase used in this study. In addition, the usefulness of tempering eggs at different developmental stages and from different species.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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