

*Full Length Research Paper*

# **Water hardness does not influence iodine-induced mortality of rainbow trout eyed eggs**

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Received 4 March, 2021; Accepted 26 May, 2021

**To evaluate the effects of water hardness on survival to hatch of eyed rainbow trout *Oncorhynchus mykiss* eggs during iodophor disinfection, hard (300 mg/L CaCl<sub>2</sub>) and soft water (20 mg/L CaCl<sub>2</sub>) solutions were used in combination with iodophor treatments of 0, 100, 200, 400, 800, and 1600 mg/L. Water hardness did not significantly influence egg survival-to-hatch. In addition, there was no significant interaction between water hardness and iodine concentration on egg survival. However, significant differences in egg survival due to iodine concentration were observed. Egg survival significantly decreased at 800 mg/L, with near complete mortality occurring at 1,600 mg/L. Rainbow trout eyed egg disinfection using iodine concentrations of 800 mg/L or greater are not recommended, regardless of water hardness (calcium) concentrations.**

**Keywords:** Eyed eggs, iodophor, disinfection, rainbow trout, water hardness, mortality.

## **INTRODUCTION**

Fish eggs are chemically disinfected to reduce the risk of introducing pathogens into fish hatcheries (McFadden, 1969; Amend and Pietsch, 1972; Piper et al., 1982; De Swaef et al., 2015). While a number of methods are used, disinfection with povidone-iodine (iodophor) is one of the most common (Zawada et al., 2014; United States Fish and Wildlife Service, 2021). Povidone has been listed as a Low Regulatory Priority drug by the U.S. Food and Drug Administration, allowing it to be used at a

concentration of 100 mg/L active iodine for 10 min on fish eggs (United States Food and Drug Administration, 2021). This treatment is effective in reducing, but not eliminating, all bacteria on the external egg membrane (Kumagai et al., 1998; Wagner et al., 2008; Barnes et al., 2009).

Increasing iodophor concentrations above the standard 100 mg/L (United States Fish and Wildlife Service, 2021) results in more complete egg disinfection (Wagner et al.,

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2012; Loch and Faisal, 2016; Bergmann et al., 2018; Huysman et al., 2018). However, higher concentrations can lead to increased egg mortality (Wagner et al., 2010, 2012; Huysman et al., 2018). Although iodine toxicity and efficacy are not generally affected by overall water chemistry (Backer and Hollowell, 2000; Fraise et al., 2004), it is possible that specific chemical components of the water used to create the treatment solutions may impact subsequent egg survival.

The presence of calcium ions in water plays an important role in the survival of freshwater fish eggs (Silva et al., 2003). Calcium influences the permeability of the egg membranes, aids in the regulation of ion loss from the tissues of the fish to the water, and plays a role in allowing or preventing the uptake of certain metals through competitive inhibition (Ingersoll, 1986; Wood et al., 1990; Bijvelds et al., 1998; Matsuo et al., 2004). While calcium, expressed as water hardness, is important during salmonid egg incubation (Brown and Lyman, 1981; Ingersoll, 1986; Wood et al., 1990; Sarkheil et al., 2014), excessive levels can be detrimental (Ketola et al., 1988).

The influence of calcium on egg survival during iodine disinfection is unknown. It is possible that higher calcium concentrations could reduce the egg mortality associated with the elevated iodine levels needed to maximize egg disinfection. Thus, the objective of this study was to evaluate the effects of low and high calcium levels, as measured by water hardness, during egg disinfection treatments using different iodine concentrations on rainbow trout *Oncorhynchus mykiss* eyed egg survival.

## MATERIALS AND METHODS

### Experimental design

Eyed rainbow trout (Shasta strain) eggs from Ennis National Fish Hatchery, Ennis, Montana, USA were used. The eggs arrived at Cleghorn Springs State Fish Hatchery, Rapid City, South Dakota, USA on 4 February 2020, where the experiment began. Six iodophor (Ovadine; Syndel, Ferndale, Washington, USA) disinfection concentrations were used: 0, 100, 200, 400, 800, and 1600 mg/L of active iodine. Each concentration was prepared using soft (20 mg/L CaCl<sub>2</sub>) and hard (300 mg/L CaCl<sub>2</sub>) water. Thus, a total of 12 treatments were used in this study: six iodine concentrations in two levels of water hardness.

### Preparation of solutions

Water hardness treatments were prepared using deionized water and CaCl<sub>2</sub>. CaCl<sub>2</sub> was chosen for its solubility and ability to disassociate completely without requiring buffers in solution thereby creating water with the desired calcium concentration without pH differences. The water solutions were prepared by adding 73.26 mg of solid CaCl<sub>2</sub> (for a 20 mg/L solution) and 1098.53 mg of solid CaCl<sub>2</sub> (for a 300 mg/L solution) to 1000 mL of deionized water. After the water hardness solutions were prepared, iodophor was added to reach the desired active iodine concentrations.

### Egg incubation

At the start of the experiment, 15 trout eggs were placed in a resealable plastic bag containing one of the twelve iodophor/water hardness solutions for 10 min. After this disinfection, the eggs were placed in another resealable plastic bag with 30 mL of water of the same calcium concentration. The bag was then filled with oxygen for 45-min transport to the incubation location. Upon arrival at the incubation location, the eggs were transferred to sterile, 9.5-cm, plastic Petri dishes containing 30 mL of water of the same water hardness and incubated at 11°C in a small (~0.05 m<sup>3</sup>) refrigeration unit (model DWC350BLPA; Danby, Findlay, Ohio, USA) using the techniques described by Barnes and Durben (2008) and Neumiller et al. (2016). Four Petri dishes of eggs were used for each of the 12 treatments (N=4; 48 total dishes).

### Data collection

Hatched fry and mortalities were recorded and removed daily, until 17 February 2020, when all eggs had either hatched or deceased. Percent survival was calculated using the following formula (Slama et al., 2021):

$$\text{Survival (\%)} = 100 \times (\text{Number of fry} / \text{Initial number of eggs})$$

### Statistical analysis

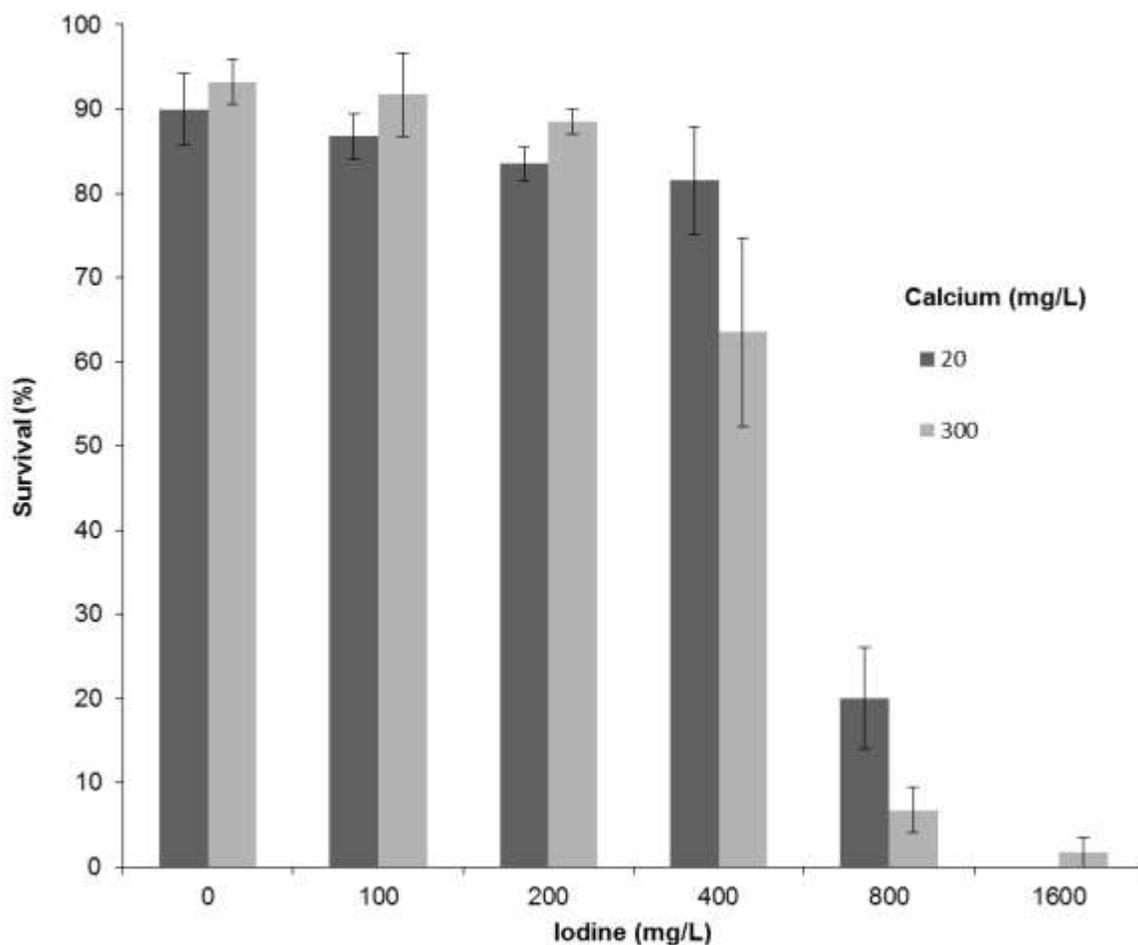
Analysis was conducted using two-way Analysis of Variance (ANOVA) in Programme R (R Core Team, 2019). Significance was predetermined at  $P \leq 0.05$ . Pairwise mean comparisons were performed using Tukey's mean comparison procedure.

## RESULTS

Water hardness had no significant ( $P > 0.05$ ) effect on egg survival at the different iodine concentrations used during egg disinfection (Figure 1;  $F = 0.34$ ,  $P = 0.57$ ). Additionally, no significant ( $P > 0.05$ ) interaction between water hardness and iodine concentration on egg survival was observed ( $F = 0.11$ ,  $P = 0.74$ ). Egg survival was significantly different among the iodine concentrations ( $F = 214.18$ ,  $P < 0.01$ ). Significantly higher egg mortality occurred at iodine concentrations of 800 and 1600 mg/L in comparison to the all other iodine levels, with near complete mortality occurring at 1600 mg/L of iodine.

## DISCUSSION

The results of this study indicate that water hardness in the form of calcium chloride did not significantly affect the survival of eyed eggs of rainbow trout subjected to a variety of iodine concentrations during a 10-min disinfection. Because this is the first experiment to examine different water hardness or calcium concentrations during iodine disinfection of fish eggs, it is difficult to determine how these results are compared to other published studies. Water hardness (as calcium) is



**Figure 1.** Mean (SE) percent survival to hatch of eyed rainbow trout eggs disinfected in six different concentrations of iodine and two levels of water hardness (as  $\text{CaCl}_2$ ).

rarely reported in studies examining the use of iodophors during salmonid egg disinfection, and only two studies have done so. Zawada et al. (2014) and Huysman et al. (2018) used only relatively hard water, ranging from 300 to 360  $\text{mL}^{-1}$  of  $\text{CaCO}_3$  on salmonid eggs. Zawada et al. (2014) evaluated iodine treatments of only 100 mg/L, whereas Huysman et al. (2018) used iodine concentrations up to 400 mg/L. However, neither study varied water hardness in relation to iodine concentration.

Rainbow trout eyed egg disinfection treatments using iodine concentrations of up to 400 mg/L for 10 min in the present study did not negatively impact survival to hatch, whereas egg mortality was higher at 800 or 1600 mg/L. Similar results were observed with iodine concentrations of up to 400 mg/L using Chinook salmon *Oncorhynchus tshawytscha* eggs (Bergmann et al., 2018; Huysman et al., 2018) and non-salmonid eggs (Dabrowski et al., 2009). In contrast, Wagner et al. (2008, 2012) reported that iodine concentrations at 1,000 mg/L did not increase mortality of rainbow trout or brown trout *Salmo trutta*

eggs, unlike this study which saw increased mortality at 800 mg/L. Wagner et al. (2010, 2012) did find a 10-min treatments of 2,000 mg/L of iodine with lethal for rainbow trout eggs. The toxicity of iodophor treatments varies among non-salmonid fish species as well (Overton et al., 2010; Stuart et al., 2010; El-Dakour et al., 2015; El-Gawad et al., 2015).

The 10-min iodophor treatment duration used in this study is the standard for treating salmonid eggs (United States Fish and Wildlife Service, 2021; United States Food and Drug Administration, 2021). Longer exposure times can increase iodine-induced egg mortality (Lahnsteiner and Kletzl, 2015) and reducing exposure time in conjunction with increased levels of iodine can mitigate egg mortality (Wagner et al., 2010). While this study showed no effect of water hardness on iodine toxicity to rainbow trout eyed eggs, the effect of water hardness during longer treatment durations is unknown. In addition, this study used only rainbow trout eggs at the eyed stage of development. Iodophor treatments are also

routinely conducted during salmonid spawning at water-hardening immediately after fertilization (United States Fish and Wildlife Service, 2021) with species-specific differences in dosage-related mortality (Chalupnicki et al., 2011). The effects of water hardness during water-hardening iodophor treatments are also unknown and cannot be inferred from this study.

In conclusion, the results of this study indicate that water hardness, at either 20 or 300 mg/L CaCl<sub>2</sub>, had no effect on the toxicity of iodophor on rainbow trout eyed eggs during 10-min treatments. In addition, iodine concentrations up to 400 mg/L can safely be used to disinfect eyed rainbow trout eggs for 10 min without a significant increase in egg mortality.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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