Survival of Arctic Grayling Eggs Water-Hardened in Various Concentrations of Iodophor

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Abstract

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Survival of Arctic grayling (Thymallus arcticus) eggs following water hardening in various concentrations of povidone iodine solution was investigated. The treatment is effective in reducing external bacterial load at the surface of the egg and is thought to reduce the risk of vertical transmission of pathogens from parent to offspring. However, in some cases, the use of iodophor during water hardening of salmon and trout eggs has resulted in significant egg losses. Treatment duration and concentrations similar to those commonly used during water hardening of eggs of other salmonid species were tested with Arctic grayling eggs. Water hardening in 50, 75, and 100 mg/L iodine resulted in significantly greater losses at eye-up than occurred when hardening in lake water. Egg mortality was highest in treatments with the strongest concentration of iodine. Additional studios are needed to determine an effective and safe method for the use of Povidone iodine as a disinfectant for freshly fertilized Arctic grayling eggs.

Introduction

Several authors have reviewed the use and efficiency of iodophors as surface disinfectants for water-hardened salmon and trout eggs. In most instances, their use has been effective without harmful effects to egg survival. (McFadden 1969; Ross and Smith 1972; Amend 1974; Alderson 1984; Fowler and Banks 1990, 1991).

The use of an iodophor compound as a disinfectant during water-hardening of salmon and trout eggs has yielded mixed results depending on iodine concentration, pH of iodophor solution, and age of post-ovulated ova. Amend (1974) reported significant losses using 100 mg/L iodine for 15 min during the water-hardening stage of rainbow trout (Oncorhynchus mykiss) eggs or when iodophor solutions dropped below pH 6.0. Alderson (1984) reported varying sensitivity of salmonid eggs to different concentrations of iodophor (75-200 mg/L iodine): mortality ranged from 0 to 83% in treatments of freshly fertilized eggs. In addition, significant differences in egg mortality were noted from parent to parent within the same iodophor treatment. Fowler and Banks (1990) tested the effects of 75 mg/L iodine for 30 min on fall chinook salmon (Oncorhynchus tshawytscha) eggs during water-hardening: mortality of eggs and fry was significantly higher than in non-treated lots. Leary and Peterson (1990) reported a 9.6% reduction in hatching success of rainbow trout eggs water hardened in a solution of 125 mg/L active iodine.

Fowler and Banks (1991) re-examined 30 min water hardening of fall chinook salmon eggs in iodophor at 50 mg/L iodine, rater than 75 mg/L iodine. Egg or fry mortality did not increase when compared with eggs water-hardened without iodophor.

Few investigations have been conducted testing povidone iodine as a disinfectant for Arctic grayling (Thymallus arcticus) However, it has been used successfully as a surface disinfectant for post water-hardened Arctic grayling eggs (W.P. Dwyer. USFWS, personal communication). Peterson (1991) attempted to water-harden grayling eggs in 75 mg/L buffered iodophor for 30 min. Treated eggs appeared to swell and burst within a few hours resulting in 100% mortality.

We examined the survival of Arctic grayling eggs at eye-up, water hardened in iodophor solutions containing 50, 75, and 100 mg/L iodine. The intent of our investigation was to find out if an egg disinfection method common in trout and salmon culture could be applied safely and effectively to the culture of Arctic grayling.

Materials and Methods

Gametes were collected from wild stock of Arctic grayling live-trapped in Axolotl Lakes near Ennis, Montana. Eggs were manually stripped from 10 females and pooled. Milt was pooled following collection from 10 males by aspiration (D.A. Erdahl, USFWS, personal communication) using a simple suction device to draw milt from each fish through a modified pipette in to a test tube. Gametes from each sex of grayling were thermally protected in insulated containers prior to fertilization. All gametes were combined into a large plastic bowl, lake water (49^{o}F. pH 8.3) added to just cover the mixture and then gently stirred. Mixed gametes were allowed to stand for 2 min before decanting. The polled lot was divided into twelve 20 mL aliquots of approximately 650 eggs each and placed in separate plastic containers (one pint capacity. BES-PAC. Webster Industries Inc., a division of Chelsea Industries Inc., Peabody, MA 01960). Povidone iodine solutions calculated at 50, 75, and 100 mg/L active iodine according to manufacturers labeled specifications (Betadine, a product of Purdue Fredric Co., Norwalk, CT 06856) were premixed with lake water. Calculated pH of all iodophor solutions tested was >7.5. Approximately 250 mL of each solution was added to three randomly selected replicates of fertilized eggs. An equivalent volume of lake water was added to each of the remaining three replicates, which served as controls. All treatment and control containers were allowed to stand for 30 min prior to decanting and refilling with fresh lake water. After rinsing, all replicates were placed in a thermally insulated container for transport to the Fish Technology Center in Bozeman, Montana. At the center, replicates of fertilized eggs were transferred into partitioned Heath^{R} trays (F.A.L./ Heath. 4540 So. Adams St., P.O. Box 9037 Tacoma, WA 98409) for incubation with water flow maintained at a rate of 4 gpm. Incubation temperature was maintained at 9^{o}C. All lots were subjected to 1200 mg/L formalin treatments for 15 min daily to prevent fungus infection. Prominent eye development was observed at 16 days post-fertilization. At eye-up all treatments ere exposed to mechanical shock. A random sample consisting of approximately 30% of the total egg column in each replicate was obtained, sorted for viability, and counted in each category. All random samples were returned to their corresponding trays to continue incubation through hatch. Data were analyzed using the General Linear Models procedure of the Statistical Analysis System (SAS 1988).

Results and Discussion

Water hardening of Arctic grayling eggs in iodophor solutions of 50, 75, and 100mg/L iodine with a calculated pH range between 7.5 and 8.2 resulted in a significant ($P<0.01$) decrease in survival to the eyed stage when compared to hardening in lake water (Table 1). Increasing concentrations of iodophor progressively decreased egg survival. Treatments of 50, 75, and 100 mg/L active iodine reduced eye-up by 10, 20 and 25% respectively compared to eggs water hardened in lake water.

Egg mortality continued following eye-up in all iodophor treatments with estimates of up to 95% loss at hatch. The non-treated eggs hatched with estimates of less than 5% post eye-up mortality. Unfortunately, we were not able to more precisely measure post eye-up survival. Based on these results, Arctic grayling eggs cannot be water hardened in iodophor treatments of similar concentration to those commonly used for salmon and trout eggs without substantial risk of severe mortality.

Further studies should be performed testing iodophor during water-hardening at reduced concentrations and contact time. Ross and Smith (1972) reported that povidone iodine compounds were effective in protecting salmonid ova against invasion of most common bacterial and fungal species in vitro when administered at 25 mg/L active iodine for 5 min. However, eggs in these studies were in the absence of blood, ovarian fluid, and mil, all which are materials that may alter the activity of iodine.

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Table-1. Survival of Arctic grayling eggs to eye-up following iodophor water hardening at various concentrations of iodine. All figures are means of three replicates. SD of eye-up % in parentheses.

Water-Hardening Medium	No. of eggs in sample	No. of eyed eggs	No. of dead eggs	Eye-up* (%)
Lake water	227	169	58	75 (4.69)
50 mg/L active iodine	254	163	91	64 (3.55)
75 mg/L active iodine	246	136	110	55 (4.82)
100 mg/L active iodine	221	113	108	50 (7.40)

*Analysis of variance for percent eye-up: effect of treatment: $P<0.01$. R-squared = 0.82, CV = 8.7