

COMMUNICATIONS

Effect of Iodophor Concentration and Duration of Exposure during Water Hardening on Survival of Atlantic Salmon Eggs

WADE A. JODUN* AND MICHAEL J. MILLARD

U.S. Fish and Wildlife Service, Northeast Fishery Center,
Lamar, Pennsylvania 16848, USA

Abstract.—Because of disease transmission concerns, field studies to evaluate the impact of water-hardening eggs at different concentrations of polyvinylpyrrolidone iodine (iodophor) for various times of exposure have rarely used untreated controls. Additionally, U.S. Fish and Wildlife Service protocol requires a subsequent post-water-hardening surface disinfection for salmonid eggs transferred between stations. The cumulative impact of this second disinfection on survival has not been fully investigated for Atlantic salmon *Salmo salar*. This study compared the percent of eye-up Atlantic salmon eggs that had been water-hardened with iodophor treatments at 50, 100, and 150 mg active ingredient/L for 30, 60, and 90 min with that of untreated controls and also examined the impact on egg survival of a second iodophor disinfection 5 h after the initial exposure. No discernable mortality resulted from the second (10-min) disinfection. Nontreated eggs had significantly greater survival than any of the iodophor-treated eggs. Contact time with the iodophor solution had the greatest impact on egg survival. When averaged over all concentrations, the decline in egg survival was significant ($P < 0.05$) when contact time increased from 30 to 60 min. Interaction between iodophor concentration and exposure time was most evident at the high (150 mg/L) concentration, with egg mortality increasing with contact time. Our study suggests that to optimize egg survival, contact with iodophor during water hardening should be no more than 30 min. If a greater disinfection efficacy is desired, an increase in iodophor concentration may be preferable to an increase in contact time.

Because of their toxicity to numerous fish pathogens, iodophors (iodine polyvinylpyrrolidones) have become widely adopted as broad-spectrum egg disinfectants (Amend and Pietsch 1972). Numerous investigations have validated iodophors as effective antimicrobial egg disinfection agents for a wide array of salmonid species (McFadden 1969; Amend and Pietsch 1972; Ross and Smith 1972; Amend 1974; Fowler and Banks 1990, 1991). Eggs of Atlantic salmon *Salmo salar* have routinely received prophylactic treatments with iodine compounds to prevent or limit the transmission of cer-

tain egg-associated viral and bacterial pathogens. Iodine compounds are widely applied as nonselective disinfectants in veterinary and laboratory facilities and when used as an egg disinfectant, are considered “low regulatory priority” by the U.S. Food and Drug Administration. This allows for the application of povidone iodine compounds during the water-hardening process without an investigational new animal drug permit or a new animal drug application. However, studies have shown that at certain concentrations, iodophor can adversely impact egg survival (Fowler and Banks 1990, 1991).

Before 1988, the standard means of subjection to iodophor disinfection within the U.S. Fish and Wildlife Service (USFWS) was immersion of previously water-hardened eggs into an active iodine solution of 100 mg/L for 10 min (Leitritz and Lewis 1976; Wood 1979; Piper et al. 1982). This surface disinfection procedure had been demonstrated effective against certain bacteria (McFadden 1969) and viruses (Amend 1974). Bullock et al. (1976) and Evelyn et al. (1986), however, pointed out the inability of this egg surface treatment to eliminate certain specific diseases such as infectious pancreatic necrosis virus and bacterial kidney disease. The inability of this egg disinfection technique to control certain pathogens, coupled with the spread of infectious hematopoietic necrosis virus, caused many aquaculturists to initiate iodine treatment of eggs at the same time as water hardening instead of treating posthardening (Chapman and Rogers 1992), attempting to limit vertical transmission of disease by allowing disinfectant to penetrate into the egg.

In 1988, USFWS Fish Health Policy and Implementation Guidelines called for all salmonid eggs to be disinfected and water-hardened in iodophor. These guidelines suggested a change from the conventional method—water hardening followed by a 10-min topical disinfection with a 100 mg/L solution—to water-hardening rainbow trout *Oncorhynchus mykiss* and chinook salmon *O.*

* Corresponding author: Wade.Jodun@fws.gov

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tshawytscha eggs in a 75 mg/L solution of active iodine for 30 min at pH 7.0 or above and to use a 100 mg/L solution for 30–60 min for all other salmonids.

A revision of USFWS policy in 1995 called for all salmonid eggs shipped from or received at Service facilities to be water hardened in active iodine at 50 mg/L for 30 min. Additionally, any lot of already water-hardened eggs received at USFWS facilities were to be rehydrated for 30–60 min and then disinfected for 10 min in 100 mg/L active iodine solution before being allowed to come in contact with water used for culture, rearing units, or equipment at the receiving station. Under this policy, salmonid eggs can potentially receive two iodophor treatments within a short time. Amend (1974) observed no apparent impact on the survival of rainbow trout eggs when subjected to multiple exposures from iodophors after their initial water hardening. However, the cumulative impact of multiple disinfections on the eggs of Atlantic salmon has not been fully investigated.

Studies to evaluate the impact on egg and fry survival of water hardening Atlantic salmon eggs in the presence of iodophor have thus far yielded inconclusive results. Hendrix and Baker (1992) reported no significant difference in the survival rates of Atlantic salmon eggs hardened in iodophor at various concentrations and contact times in comparison with those treated conventionally with 100 mg/L iodine for 10 min after water hardening. However, although no significant difference in survival was observed, they noted a trend towards less egg survival as the immersion time in iodophor during water hardening increased. Additionally, the risk of disease transmission prevented the use of any untreated controls in those experiments. In this study, we used untreated controls to more fully assess the consequences of water-hardening Atlantic salmon eggs in iodophor. We also evaluated the impact on egg survival of a second iodophor disinfection of water-hardened eggs.

Methods

Source of gametes.—Connecticut River F₁ Atlantic salmon, held as broodstock at the Northeast Fishery Center, Lamar, Pennsylvania, served as the source of gametes for the investigation. Eggs were obtained by manually stripping four females. Eggs from different females were stripped into separate bowls, egg quality was assessed visually, and poor-quality eggs (broken, hemorrhagic, overripe) were rejected. Care was taken to avoid exposing newly stripped eggs to water, which has the potential to

cause closure of the micropyle, thus resulting in an artificially low or incomplete fertilization. Precautions were also taken to shield eggs from exposure to bright light, given its potential detrimental effects (Piper et al. 1982). Milt was collected from six Connecticut River F₁ males. Individual milt specimens were maintained in separate plastic bags (Whirl-Pak; NASCO, Fort Atkinson, Wisconsin) and kept on ice until they could be examined microscopically for viability (approximately 45 min later). Motility of each of the six lots of milt was determined by placing a small amount of sperm in contact with ovarian fluid on a slide and examining microscopically. One nonmotile lot was discarded.

Newly stripped eggs were transported in ovarian fluid to the incubation area, where they were pooled, thoroughly mixed, and held in large bowls to await fertilization. Aliquots of milt from the males were pooled. Eggs were then fertilized with 2 mL of pooled milt. After addition of the pooled milt, the gametes were gently stirred to ensure thorough mixing and allowed to stand 2–3 min to facilitate complete fertilization. Excess milt was drained off and aliquots of approximately 300 eggs each were placed in 15-cm-diameter small plastic bowls with a maximum capacity of approximately 425 mL. Egg enumeration was based on the volumetric determination of eggs per liter.

Iodophor treatments.—Stock solutions of pre-buffered iodophor (Argentyn[®]; Argent Chemical Laboratories, Redmond, Washington) were used to prepare concentrations containing 50, 100, and 150 mg iodine/L as active ingredient. Individual bowls were then randomly assigned one of the four concentration treatments (0, 50, 100, or 150 mg active iodine/L) and one of three exposure treatments (30, 60, or 90 min). The egg aliquots were immersed in approximately 400 mL of the iodophor solutions, gently stirred, and allowed to water-harden undisturbed in the static solution for the assigned exposure times. Each treatment combination was performed in triplicate, resulting in 36 experimental units. After treatment, the disinfectant was drained, the egg lots were rinsed in freshwater, and each lot was transferred into a randomly assigned location in partitioned Heath trays (F.A.L./Heath Techa Co., Tacoma, Washington).

To simulate a second iodophor treatment, which might be received by eggs being transferred between stations, a duplicate set of 36 egg lots received a subsequent disinfection. Five hours after the initial iodophor treatment, these egg lots were removed from the incubators, immersed for 10 min

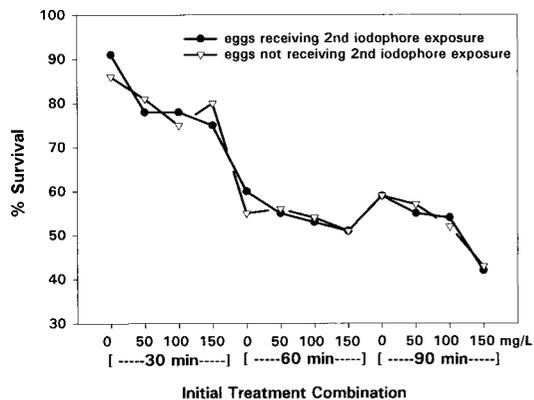


FIGURE 1.—Mean survival of Atlantic salmon eggs treated and not treated with a 10-min second iodophor disinfection with a 100 mg/L solution, after identical initial treatments with three iodophor concentrations, plus an untreated control, and three contact times. The ANOVA results showed no significant difference ($P > 0.80$) between mean survival of secondarily treated and nontreated eggs.

in 100 mg/L active iodine, and returned to the incubators. To ensure identical handling effects, the initial single-exposure egg lots were also removed from the incubators and immersed in water for 10 min.

During incubation, fresh water was introduced at the rate of 15.2 L/min. All eggs received a 15-min flow-through treatment of 1,500 mg/L formalin solution every other day, beginning on the second day of incubation and continuing until just before hatching. Water temperature, pH, and formalin treatments were recorded daily. Mortalities were enumerated and removed periodically during incubation. Once they attained eye-up, all egg lots were mechanically shocked by striking the partitioned incubator trays several times against the surface of water in a rectangular tank filled to a depth of 0.5 m. Shocked eggs were then returned to the incubator trays for approximately 3.5 h. Unfertilized eggs (those that appeared opaque from the rupture of the egg membrane) were then removed manually through the use of a bulb and pipette and counted. The remaining eyed eggs were then enumerated.

Statistical analyses.—Postshock survival was calculated as the proportion of total eggs, per egg lot, that remained viable (eyed) at the conclusion of the trials. The data were analyzed as a factorial design, with iodophor concentration at first disinfection, time of immersion at first disinfection, and secondary disinfection as the main effects. Initial analyses showed that the secondary disinfection

TABLE 1.—Mean percent survival and 95% confidence intervals (CI) for Atlantic salmon eggs treated with 50, 100, and 150 mg/L iodophor solutions concentrations and an untreated control (0 mg/L), and 30-, 60-, and 90-min contact times. Treatment means not followed by a common letter are significantly different ($P \leq 0.05$) based on Tukey-adjusted means comparisons.

| Treatment | Survival (%) | |
|-----------------------------|--------------|-----------|
| | Mean | CI |
| Iodine concentration (mg/L) | | |
| 0 | 68.3 z | 66.6–70.0 |
| 50 | 63.7 y | 62.1–65.5 |
| 100 | 61.2 y | 59.5–62.9 |
| 150 | 55.5 x | 55.5–58.9 |
| Contact time (min) | | |
| 30 | 80.7 z | 79.2–82.1 |
| 60 | 54.5 y | 53.0–56.0 |
| 90 | 52.7 y | 51.2–54.2 |

treatment elicited no discernable influence on mean survival, and a subsequent test of equality of variances between secondary disinfection groups showed no significant difference ($P > 0.8$). As such, this effect was removed from the final analyses and the data were pooled to increase statistical power. Analysis of variance (ANOVA) and a Tukey-adjusted means comparisons were used to test for differences among treatment main effects. Percent values were not transformed because most values were between 50% and 75% (Zar 1999). All analyses were performed with SAS software (SAS Institute 1989).

Results

Main Effects

A second exposure to 100 mg/L iodine for 10 min at 5 h after the initial water hardening in iodophor had no discernable effect ($P > 0.80$) on egg survival (Figure 1). The mean survival of lots subjected to the second iodine exposure was 62.7%, compared with 62.6% for the lots receiving no secondary treatment.

With respect to the initial exposure to iodophor during water hardening, egg survival significantly decreased as exposure time increased from 30 to 60 min (Table 1). No difference was observed between the 60- and 90-min treatments when pooled over concentrations. Mean egg survival also decreased significantly as the concentration of iodophor increased. Indeed, the most survival was observed in the control group, which received similar handling but no iodine treatment (Table 1). An inverse relationship was observed between survival and iodine concentration (Figure 2).

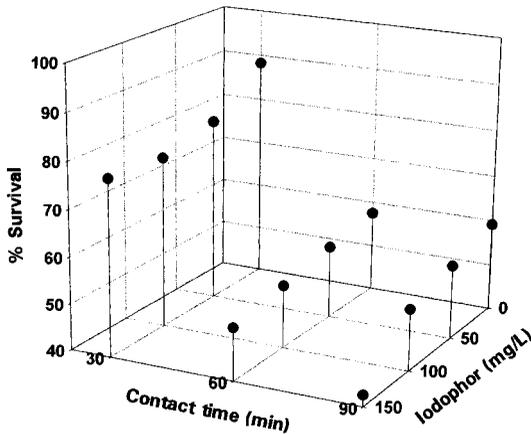


FIGURE 2.—Mean survival of Atlantic salmon eggs treated with 0, 50, 100, or 150 mg iodophor/L for 30, 60, or 90 min.

Interaction Effects

The concentration \times time interaction was significant in the ANOVA ($P < 0.01$), and inspection of the cell means provided a clear indication of the nature of the interaction without formal testing of all possible pairwise combinations. Survival decreased with incremental increases in both exposure time and iodine concentration (Figure 2). In no case did treatment with iodine at any concentration increase survival over that of the nontreated control group for a given exposure time.

Discussion

Our results suggest that increasing the concentration or contact time of iodophor elicits progressively greater egg mortality. Eggs of other salmonids have exhibited a similar decline in survival in the presence of iodine during the water-hardening stage. Leary and Peterson (1990) reported a 9.6% decline in survival of rainbow trout eggs hardened in a 125 mg/L iodophor solution compared with that of untreated, water-hardened controls. Studies with rainbow trout (Amend 1974) and coho salmon *Oncorhynchus kisutch* (Evelyn et al. 1986) indicated that water-hardening eggs in the presence of iodophor impacted survival more adversely than did treating posthardened eggs. While testing iodine disinfection during the water-hardening process for chinook salmon eggs, Fowler and Banks (1990, 1991) reported no significant increase in egg mortality above that of untreated eggs when water-hardened in an iodophor solution of 50 mg/L for 30 min. However, when a concentration of 75 mg/L was used for the same duration,

mortalities of eggs and fry were significantly more than in untreated lots. Brown and Shrable (1994) observed a similar pattern while testing the effect of water hardening the eggs of Arctic grayling *Thymallus arcticus* with various iodine concentrations for 30 min. The percent of eye-up eggs among their controls (75%) was significantly ($P < 0.05$) greater than in those eggs treated at 50 mg/L (64%), 75 mg/L (55%), and 100 mg/L (50%). Evelyn et al. (1986) also found that increasing contact time from 30 to 60 min in 250 mg/L active iodine solution decreased the survival of coho salmon eggs.

Our data suggest that contact time exerts the largest detrimental influence on survival of eggs treated with iodophor during the process of water-hardening Atlantic salmon eggs. We conclude that restricting iodophor treatments during water-hardening to 30 min or less will result in decreased mortality. If greater disinfection efficacy is desired, increasing iodophor concentration may be preferable to increasing contact time. However, our experimental design does not allow us to attribute the observed mortality solely to iodophor disinfection. Great care was taken to ensure all lots were subjected to identical handling procedures. Therefore, one explanation may be that the losses were a result of an increased sensitivity by the eggs to mechanical shock at some time beyond 30 min postfertilization. Jensen and Alderdice (1983) found that sensitivity of coho salmon eggs to mechanical shock appeared to increase rapidly, peaking at about 15 min after activation. Subsequent investigation (Jensen and Alderdice 1989) demonstrated that the shock sensitivity of eggs varies not only among species but also among particular stages of egg development. Although not measured in this study, low-oxygen conditions in the microhabitat surrounding small numbers of eggs during the disinfection process could impact egg survival.

In most artificial propagation programs, the egg loss caused by iodophor treatment is an acceptable compromise, given the value of the treatment as a means of controlling disease transmission. The results also suggest that no excessive mortality should result from agency disinfection protocols that require a second disinfection at 100 mg/L for 10 min on arrival at another facility. Additional investigations are needed to determine the species-specific impact on egg survival imparted by the extra handling necessary to treat with iodophor; to assess whether even shorter exposure times (5–10 min) could effectively control known salmonid pathogens; and to assess the extent to which water volume, density, and oxygen content within the various static treatments influence survival.

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