IODOPHOR DISINFECTION OF FISH EGGS

Disclaimer: *Mention of specific brands or manufacturers does not warrant endorsement by the U.S. Fish and Wildlife Service. Any comparable reagent may be substituted in this protocol, if operation and performance are deemed comparable to items specified.*

Introduction

This chapter describes the use of iodophor solutions (polyvinylpyrrolidone iodine) for the surface disinfection of salmonid eggs. While these protocols are directed at salmonid fishes, iodophor egg disinfection (IED) of epibiotic pathogens has been reported to increase survival in a variety of teleost eggs such as sturgeon, grouper and halibut (Bouchard and Aloisi 2002, Tendencia 2001, Bergh and Jelmert 1996). Iodophor egg disinfection has been practiced since the 1970's to reduce the transmission of viral and bacterial pathogens associated with the egg, coelomic fluid, or milt. It is an established hatchery practice to mitigate the disease threat of vertically transmitted pathogens. A 10-60 min bath in 50-100 mg/L active iodine solution is the standard procedure performed both on recently-fertilized eggs (green egg) during the water hardening step and with eyed eggs (Amend 1974, Piper et al. 1986, Wood 1979). Previous U.S. Fish and Wildlife Service (Service) Fish Health Policies directed the use of IED for all salmonid species during the water-hardening step and prior to receipt of eyed-eggs (USFWS 1995). Recent field studies by Cipriano et al. (2001) demonstrated the efficacy of the Service IED protocol against egg-associated *Aeromonas salmonicida*. The U.S. Food and Drug Administration considers IED, as described in the Service protocol listed below, a low regulatory priority drug (Wedemeyer 2001).

The range of recommended active iodine concentration for IED is reflective of the different sensitivities of various salmonid species and stocks. Hatchery personnel must first determine sensitivity of the eggs for a given fish stock to water-hardening in 50, 75 and 100 mg/L iodophor prior to adoption of IED on a production level. The pH of the solution must be monitored and maintained between 7.0 and 7.5 for optimal results.

Recommended usage

All salmonid eggs shipped from or received at Service facilities shall be disinfected in 50-100 mg/L iodine for 30 min during the water-hardening process. Allowing an appropriate time for fertilization, eggs should be washed in pathogen-free water at a temperature similar to that experienced by the brood fish. The objective of this step is to reduce the contaminate effects of semen, blood, and coelomic fluid on IED. It is essential that iodophor not come in contact with water used in the fertilization step.

Eggs received at a Service facility shall be disinfected before they are allowed to come into contact with fish cultural water, rearing units, or equipment at the receiving station. Eggs being disinfected upon receipt shall be placed in water for 30-60 min. before adding iodine compound to replenish water loss that occurs in the eggs during shipping. Eyed eggs shall be disinfected in a solution providing 100 mg/L of active iodine for 10 min. Care must be taken to avoid treatment of hatched fry as they are extremely sensitive to iodophor exposure. Iodine can be neutralized by the addition of sodium thiosulfate solutions (e.g. 1.5 g/L) until a change to a clear coloration indicates neutralization (Alaska Department of Fish and Game 1988).

Hatchery staff must monitor the color of the solution over the treatment period. Iodine concentrations below 20 mg/L appear a light yellow and indicate that IED is not meeting minimum standards. The target pH of treatment should be in the range of 7.0-7.5. The use of pathogen-free water at the appropriate temperature for egg fertilization, rinsing, and preparation of iodophor solution is an obvious factor in disease control. Additionally, the use of pathogen free water (well-water, UV filtered) for egg incubation will greatly enhance the effectiveness of IED.

Specific protocols

1. If water used during disinfection is below 100 mg/L total alkalinity, the disinfection solution should be buffered by adding sodium bicarbonate ($NaHCO_3$) at a level of 0.01 percent to prevent egg toxicity effects of low pH drift (< 6.0).

2. Listed below are quantities of 1.0 percent iodine or sodium bicarbonate needed to obtain the solutions required by these guidelines. Quantities used in these examples are based on the following equivalencies: 3.78 L = 1 gallon, 28.32 L = 1 cubic foot and 454 g = 1 pound.

    a. To get 100 mg/L active iodine solution, add either:
        37.8 mL organic iodine solution to 3.78 L H_2O or
        283.2 mL organic iodine solution to 28.32 L H_2O or
        4.54 mL organic iodine solution to 454 g H_2O.

    b. To get 75 mg/L active iodine solution, add either:
        28.50 mL organic iodine solution to 3.78 L H_2O or
        212.40 mL organic iodine solution to 28.32 L H_2O or
        3.4 mL organic iodine solution/454 g H_2O.

    c. To get 50 mg/L active iodine solution, add either:
        18.9 mL organic iodine solution to 3.78 L H_2O or
        141.6 mL organic iodine solution to 28.32 L H_2O or
        2.27 mL organic iodine solution to 454 g H_2O.

    d. To get 0.01 percent sodium bicarbonate, add either:
        0.378 grams $NaHCO_3$ to 3.78 L H_2O or
        2.83 grams $NaHCO_3$ to 28.32 L H_2O or
        0.045 grams $NaHCO_3$ to 454 g H_2O.

Review of iodophor disinfection

Mechanism of iodophor disinfection

Four forms of iodine can be present in aqueous solutions; (I_2) elemental iodine, (HIO) hypoiodus acid, (I_3) periodide, and (IO_3) iodate ion. Both iodine and hypoiodus acid are germicidal. As pH increases from 6.0 to 8.0, the percent of iodine drops from 90% to 12% while hypoiodus acid increases from 10% to 88%. Maintaining pH between 7.0 and 7.5 optimizes the balance between these two ion species. Microbial killing during IED occurs due to either oxidation or halogenation of the microorganism. Brandrick et al. (1967) reports 11-16 % of the available iodine in an iodophor solution will bind to bacterial cell walls with the subsequent oxidation of elemental iodine to iodide. Low iodine concentrations can rapidly kill most fish pathogens when they are suspended in distilled water. IHNV is reported to be inactivated by 99.9% after only a 7.5 second contact with 0.1 mg/L iodine (Batts et al. 1991). Ross and Smith (1972) report that nine species of bacteria and two species of fungus pathogenic to fish were effectively killed by a 5 min exposure to 25 mg/L iodine.

Toxicity to eggs

Fowler and Banks (1990) report that IED (75 mg/L for 30 min) of fall-run Chinook eggs at water-hardening resulted in 2-3% higher mortality at the eyed egg stage than untreated controls. Subsequent work by these same authors demonstrated that a 30 min treatment at 50 mg/L did not produce significant mortality (Fowler and Banks 1991). Grayling eggs (*Thymallus arcticus*) are reported to be quite sensitive to IED (50-100 mg/L) during water hardening. Brown and Shrable (1994) report an 11, 20 and 25 % reduction in eye-up rate for eggs water-harden in 50, 75 and 100 mg/L for 30 min, respectively. Alderman (1984) cautioned that individual female egg quality can greatly affect IED data and concluded that 10 min at 100 mg/L iodine treatments did not cause significant egg mortality in rainbow trout.

Limitations

Iodophor egg disinfection will act to reduce the probability of egg surface pathogen transmission, but does not completely kill all microbes. A numbers of factors act to reduce the effectiveness of IED such as the presence of the pathogen within the yolk of the egg (inability of iodophor to contact pathogen), masking effect of organic matter on the egg, improper pH or iodine concentration, or specific resistance characteristics of the pathogen. Protein associated with the egg surface (e.g., blood, semen or coelomic fluid) must be washed off prior to IED. There is rapid decrease in titratable iodine during IED of washed salmonid eggs. Over a 10 min water-hardening period, up to 90% of the original titratable iodine can be lost (unpublished data, California- Nevada Fish Health Center data on washed chinook salmon eggs water-hardened in 30 and 60 mg/L iodophor). This phenomenon requires the use of iodophor concentrations higher than those reported for fish pathogens suspended in water for IED efficacy.

Pathogen	Treatment (mg/L; duration)	Notes	Reference
Cytophaga psychrophilia	50-1000; 15 min	WH /EE	Kumagai et al. 1998
Loma salmonae	100-200; 25 min	spores	Shaw et al. 1999
IPNV	25-200; 45 min	WH	Dorson et al. 1997
IHNV	100; 10 min	EE/SP	Goldes and Mead 1995
R. salmoninarum	100; 10 min	WH	Elliott et al. 1991
R. salmoninarum		WH	Evelyn et al. 1996
Notes: EE = eyed egg stage; SP = seeded pathogen; WH = water hardening stage			

The proper ratio of egg mass to iodophor solution and gentle circulation will improve IED efficacy. Chapman and Rogers (1992) report iodine concentration within the egg mass was significantly lower than the egg mass surface if the iodophor solution was not circulated. Up to 70% of the activity was lost within the egg mass in un-circulated treatments. Circulation during IED did not improve efficacy when small number of eggs were experimentally treated (Goldes and Mead 1995). The primary objective appears to be sufficient contact of all egg surfaces by the solution. Groberg (1990) recommends a 15 min treatment period at 50-100 mg/L when the ratio of iodophor to egg volume is kept at 1:1. Water-hardening in iodophor for up to an hour can occur if movement of the fertilized eggs could result in significant trauma.

Workers have reported that some species of bacteria significant to hospitals are resistant to concentrated povidone-iodine solution (10%) yet are killed in 100x dilutions of the concentrate (Berkelman et al. 1982). The glycocalyx cover of *Pseudomonas aeruginosa* appeared to protect it from iodophor disinfection of contaminated PVC pipes (Anderson et al. 1990). Similarly, Ross and Smith (1972) report a low number of Pseudomonas bacteria surviving a 25 mg/L treatment for 5 min.

The scientific literature contains several publications providing information about the survival of fish pathogens following IED procedures. These studies have been summarized in the following table.

Human safety

A number of safety steps must be followed to ensure worker safety. When performing IED at the recommended concentrations, rubber gloves, rain suits or aprons, rubber boots and some form of eye protection should be worn. Eye protection can be either be safety glasses or goggles to prevent eye injury from splash. These protective measures are particularly important when handling the 1% concentrated iodophor solutions.

Iodophor solutions should never be atomized for any application due to the documented respiratory irritation and hypersensitivity problems (Alaska Department of Fish and Game

1988). Use of dilute solutions in well-ventilated location should avoid aerial exposure to levels greater than the 0.1 mg/L permissible exposure limit (Alaska Department of Fish and Game 1988). If these conditions are not present, the user should wear a respirator with an iodine cartridge.

Other uses

The primary industrial use of iodophor is for disinfection of clean surfaces (no organic residue) or utensils. Iodophor disinfection of nets, boots and tools are an important element in the biosecurity of hatchery operations. A recommended exposure to 50-200 mg/L for 5-30 min has been recommended for such functions in the hatchery (Alaska Department of Fish and Game 1988, Wedemeyer 2001). Some hatcheries now employ 0.2-0.4 mg/L iodophor treatments to control fungus on eggs. While comparable to formalin, iodine treatment results in little decomposition of the egg shells after hatch. Both California Department of Fish and Game and Washington Department of Fish and Wildlife employ iodophor treatment of eyed eggs to control external fungus in locations where formalin use is inappropriate (Hatfield 1991, personal communication Mel Willis CDFG).

References

Alaska Department of Fish and Game. 1988. Safer chemical use in Alaskan Aquaculture, Version 1, 62 pp, Division of Fisheries Rehabilitation, Enhancement and Development, P.O. Box 3-2000, Juneau AK 99802.

Alderman, DJ. 1984. The toxicity of iodophors to salmonid eggs. *Aquaculture* **40**:7-16.

Amend, DF. 1974. Comparative toxicity of two iodophors to rainbow trout eggs. *Transactions of the American Fisheries Society* **103(1):**73-78.

Anderson RL, BW Holland, JK Carr, WW Bond and MS Favero. 1990. Effect of disinfectant on Pseudomonads colonized on the interior surface of PVC pipes. *American Journal of Public Health* **80**(1):17-21.

Batts WN, ML Landolt and JR Winton. 1991. Inactivation of Infectious Hematopoietic Necrosis Virus by low levels of iodine. *Applied and Environmental Microbiology* **57(5):**1379-1385.

Bergh O, and A Jelmert. 1996. Iodophor disinfection of eggs of Atlantic halibut. *Journal of Aquatic Animal Health* **8**:135-145.

Berkelman RL, BW Holland and RL Anderson. 1982. Increased bactericidal activity of dilute preparations of povidone-iodine solutions. *Journal of Clinical Microbiology* **15(4):**635-639.

Bouchard HJ and DB Aloisi. 2002. Investigations in concurrent disinfection and de-adhesion of lake sturgeon eggs. *North American Journal of Aquaculture* **64**:212-216.

Brandrick AM, JM Newton, G Henderson and JA Vickers. 1967. An investigation into the interaction between iodine and bacteria. *Journal of Applied Bacteriology* **30(3):**484-487.

Brown DR and JB Shrable. 1994. Survival of Arctic grayling eggs water-hardened in various concentrations of iodophor. *The Progressive Fish-Culturist* **56:**262-264.

Chapman PF and RW Rogers. 1992. Decline in iodine concentration of iodophor during water hardening of salmonid eggs and methods to reduce this effect. *The Progressive Fish-Culturist* **54(2):**81-87.

Cipriano RC, BM Novak, DE Flint and DC Cutting. 2001. Reappraisal of the federal fish health recommendation for disinfecting eggs of Atlantic salmon in iodophor. *Journal of Aquatic Animal Health* **13:**320-327.

Dorson M, P Rault, P Haffray and C. Torhy. 1997. Water-hardening rainbow trout eggs in the presence of an iodophor fails to prevent the experimental egg transmission of Infectious Pancreatic Necrosis Virus. *Bulletin of European Association of Fish Pathologists* **17(1):**13-16.

Elliott DG, RJ Pascho and GL Bullock. 1991. Developments in the control of bacterial kidney disease of salmonid fishes. *Diseases of Aquatic Organisms* **6(3):**201-215.

Evelyn TPT, L Prosperi-Porta, and JE Ketcheson. 1986. Persistence of the kidney-disease bacterium, *Renibacterium salmoninarum* in coho salmon, *Oncorhynchus kisutch* (Walbaum), eggs treated during and after water-hardening with povidone-iodine. *Journal of Fish Diseases* **9:**461-464.

Fowler LG and JL Banks. 1990. Iodophor toxicity to eggs and fry of fall chinook salmon. *The Progressive Fish-Culturist* **52:**176-178.

Fowler LG and JL Banks. 1991. A safe level of iodophor for treating eggs of fall chinook salmon during water-hardening. *The Progressive Fish-Culturist* **53(4):**250-251.

Goldes SA and SL Mead. 1995. Efficacy of iodophor disinfection against egg surface-associated Infectious Hematopoietic Necrosis Virus. *The Progressive Fish-Culturist* **57:**26-29.

Groberg WJ. 1990. Water-hardening salmonid eggs in iodophor. Pacific Northwest Fish Health Protection Committee. Informational Report 2.

Hatfield DG. 1991. A comparison of PVP iodine and formalin as a means of fungus control in coho salmon eggs. pp 132-135, **In:** *Proceedings of the 42nd Northwest Fish Culture Conference*; Redding, California.

Kumagai A, K Takahashi, S Yamaoka and H. Wakabayashi. 1998. Ineffectiveness of iodophor treatment in disinfecting salmonid eggs carrying *Cytophaga psychrophila*. *Fish Pathology* **33(3):**123-128.

Piper RG, IB McElwain, LE Orme, JP McCraren, LG Fowler, and JR Leonard. 1986. *Fish Hatchery Management*, Washington, D.C., U. S. Fish and Wildlife Service.

Ross AJ, and CA Smith. 1972. Effect of two iodophors on bacterial and fungal fish pathogens. *Journal of Fisheries Research Board of Canada* **29:**1359-1361.

Shaw RW, ML Kent and ML Adamson. 1999. Iodophor treatment is not completely efficacious in preventing *Loma salmonae* (Microsporidia) transmission in experimentally challenged chinook salmon, *Oncorhynchus tshawytscha* (Walbaum). *Journal of Fish Diseases* **22:**311-313.

Tendencia, EA. 2001. Effect of iodine disinfection on the bacterial flora and hatching rate of grouper, *Epinephelus coioides*, eggs at the cleavage and eyed stages. *Bulletin of the European Association of Fish Pathologists* **21(4):**160-163.

U.S. Fish & Wildlife Service. 1995. "Fish Health Policy." *U.S. Fish and Wildlife Service Manual*, Section 713 FW 1-4.

Wedemeyer GA. 2001. Editor. *Fish Hatchery Management*, 2nd edition. American Fisheries Society; Bethesda, Maryland.

Wood JW. 1979. *Diseases of Pacific Salmon; Their Prevention and Treatment*. State of Washington, Department of Fisheries, Hatchery Division.