Appendix V

*BKD Management
and Iodophor Disinfection Guidelines*

California Hatchery Review Project
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1.   BKD Management Strategy

The California HSRG recommends that managers implement a BKD control strategy for hatchery programs where BKD has proved a recurring problem. The strategy should include culling (destroying) eggs/progeny from hatchery- and natural-origin brood that are found to be infected with the BKD agent (*Renibacterium salmoninarum*), detected by current American Fisheries Society "Bluebook" methods. However, because brood fish with high levels of the BKD agent are more likely to transmit the agent to their progeny than brood with lesser levels of the agent, the culling of eggs/progeny from infected brood fish, should, at the very least, be applied to those with high levels of the BKD agent. In addition, in programs using ESA-listed natural-origin brood fish, the culling of their eggs/progeny may, at the managers' discretion, be dispensed with. However, the ESA-listed broodstock should be injected, pre-spawning, with an appropriate antibiotic (preferably, azithromycin at 40 mg/kg fish), and the resulting eggs should be surface-disinfected with an iodophor. All pre-spawning brood injections may be limited to females, ESA-listed or otherwise.

Eggs and hatchlings derived from broodstock found to be heavily infected with the BKD agent should be incubated and reared in isolation from those obtained from broodstock with no or lesser levels of the BKD agent. In addition, the hatchlings should be reared at the lowest possible densities (below current standards), and, at the first signs of infection with the BKD agent, they should be treated with an appropriate chemotherapeutic drug (e.g., orally administered erythromycin (100 mg/kg fish) for 28 days, or Florfenicol (15mg/kg fish) for 10 days). The treatment should be repeated if there is evidence that the BKD agent has persisted in the hatchlings.

2.   Iodophor Disinfection of Fish Eggs

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

2.1   Introduction

This section 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.

2.2 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.

2.2.1 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 H20 or

283.2 mL organic iodine solution to 28.32 L H20 or
4.54 mL organic iodine solution to 454 g H20.

b. To get 75 mg/L active iodine solution, add either:
28.50 mL organic iodine solution to 3.78 L H20 or
212.40 mL organic iodine solution to 28.32 L H20 or
3.4 mL organic iodine solution/454 g H20.

c. To get 50 mg/L active iodine solution, add either:
18.9 mL organic iodine solution to 3.78 L H20 or
141.6 mL organic iodine solution to 28.32 L H20 or
2.27 mL organic iodine solution to 454 g H20.

d. To get 0.01 percent sodium bicarbonate, add either:
0.378 grams NaHC0$_3$ to 3.78 L H20 or
2.83 grams NaHC0$_3$ to 28.32 L H20 or
0.045 grams NaHC0$_3$ to 454 g H20.

2.3   Review of Iodophor Disinfection

2.3.1   Mechanism of Iodophor Disinfection

Four forms of iodine can be present in aqueous solutions; (h) elemental iodine, (HIO) hypoiodus acid, (1]) periodide, and (103) 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.

2.3.2   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.

2.3.3   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.

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.

Table VIII-1. Summary of iodophor treatments for specific pathogens as noted in scientific literature.

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 Mean 1995
R. salmoninarum	100; 10 min	WH	Elliot et al. 1991
R. salmoninarum		WH	Evelyn et al. 1996

Notes: EE = eyed egg state, SP = seeded pathogen, WH = water hardening stage.

2.3.4 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 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.

2.3.5 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).

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