Effect of Povidone Iodine on TAM Viability

When fish eggs are collected from wild broodstocks and other sources outside the hatchery, the potential for bringing a pathogen into the hatchery is a serious concern to hatchery managers. As a precaution, povidone iodine (1-ethyenyl-2-pyrrolidinone homopolymer and 1-vinyl-2-pyrrolidinone polymer iodine complex) is used routinely for disinfection of fish eggs in water that potentially carry pathogens. Betadine**7** or PVP-Iodine is a commercial product that is 10% povidone-iodine (1% active iodine). The concentration of this stock solution typically used to disinfect salmonid eggs is 1% or 100 ppm of active iodine (Ross and Smith 1972; Amend 1974). While this concentration has been shown to be effective for controlling the majority of external bacteria and viruses in *in-vitro* tests (Amend and Pietsch 1972; Ross and Smith 1972; Chapman and Rogers 1992; Goldes and Mead 1995; Kumagai et al. 1998), the effect on *Myxobolus cerebralis* has not been tested. Vital staining has been useful for many applications, including the testing the viability of *M. cerebralis* (Markiw 1992).

A series of tests were conducted on triactinomyxons (TAMs), the infective stage of the salmonid parasite *Myxobolus cerebralis,* to determine the concentration of iodine needed to kill them. For each concentration, three to six replicate tests were conducted. For each test, 2 mL of the test iodine solution were mixed with 2 mL of TAM stock solution in a test tube and left at room temperature (15-20 C) for 10, 30 or 60 min. The pH of the mix was 6.5. A minute or two before the time was up, the mixed solution was poured into a 10 um mesh filter to start filtering. At the allotted time, the filter retentate was rinsed with 20 mL of hatchery well water. This process took several minutes, after which 100 uL aliquots of retentate were transferred to 3-4 microscope slides. The slides were subsequently stained with 50-75 uL each of propidium iodide (52 mg/L) and fluorescein diacetate (100 uL of 5 mg/mL stock solution diluted with 8.3 mL hatchery well water). Control slides were made from the TAM stock solution and stained as noted above. The concentration of the iodine stock solution was verified with a commercial colorimetric test. The same stock solution was used for all tests.

After incubation in a refrigerator at 4-7EC for at least 45 min, the TAMs were observed by epifluoresence microscopy. The TAMs were classified as either red (dead), green (viable), or red and green (possibly viable).

The results of the iodine tests are presented in Table 1. Povidone-iodine concentrations of 50% or 5,000 ppm of active iodine for an hour were required to kill greater than 99% of the TAMs. This is 50 times the concentration typically recommended for treatment of fish eggs for bacterial and viral disinfection (McFadden 1969). These results should be confirmed by attempting to infect fish with treated TAMs.

Clearly, higher concentrations of iodine are needed to adequately disinfect incoming water for *M. cerebralis*. The question becomes AHow high can we safely go?@ Amend (1974) tested the toxicity of Betadine and Wescodyne**7** (1.6% active iodine in the form of 9.1% polyethoxy polypropoxy polyethoxy ethanol-iodine complex, 8.74% nonylphenoxypolyethanoxyethanol iodine complex and 82% inert ingredients) to rainbow trout eggs. Amend (1974) found that toxicity was dependent upon pH and the stage of development of the eggs. At pH 6.9, the LC_{50} for active iodine was 1480 ppm in a 15 min treatment or 1050 ppm in a 60 min treatment of eyed eggs. If the solution was buffered, the LC_{50} of eyed eggs was increased to greater than 2000 ppm at either pH 7.0 or 8.0.

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Povidone-iodine concentration % (ppm active iodine)	Duration (min)	Dead (%)	Viable (%)	Possibly viable (%)	range of TAM numbers per replicate (n)
0.0 (0.0)		1.5	91.2	7.3	10-100 (24)
1.0 (100)	10	64.3	16.5	19.2	82-100 (3)
2.5 (250)	10	67.2	25.4	7.4	76-104 (4)
5.0 (500)	10	66.3	11.7	22.0	100-100 (3)
50.0 (5,000)	10	76.5	9.3	14.2	100-160 (3)
50.0 (5,000)	30	90.7	8.8	0.4	70-102 (3)
50.0 (5,000)	60	99.3	0.5	0.2	91-117 (6)

Table 1. Mean percentage of non-viable, viable, and possibly viable triactinomyxons after exposure to various concentrations of povidone-iodine. Iodine concentrations are given as a percentage of the commercial povidone-iodine stock solution (10% povidone-iodine) and in active iodine concentration in ppm (given in parentheses). Control values were pooled.

When eggs were water hardened in iodine, 25 ppm of iodine was safe, but 100 ppm resulted in significant mortality. Leary and Pederson (1988) noted reduced survival in rainbow trout eggs water hardened in 1.24% Betadine buffered to pH 8.0. If eggs were allowed to water harden for 30 min, Amend (1974) reported no significant impacts on egg mortality, hatchability, or abnormalities at concentrations of 25, 100, or 200 ppm iodine. McFadden (1969) noted that up to 2.5% povidone iodine for 10 min was not toxic to eyed rainbow trout eggs, but concentrations of 3, 4, and 5% (300-500 ppm iodine) resulted in eggs surviving less than 24 h (no pH given). Alderman (1984) noted that concentrations of iodine from 75 to 200 ppm at pH values of 6.5, 6.75, or 7.5 for 10 min were safe for eyed Atlantic salmon *Salmo salar* eggs. For eyed rainbow trout eggs, Alderman (1984) tested concentrations of 50 to 4,000 ppm iodine for 10 min at pH levels from 3 to 8 and found that the LD_{25} was about 800 ppm at pH 6.0 and in excess of 3000 ppm at pH 7.0. For freshly fertilized rainbow trout eggs, Alderman (1984) found that mortality was also highly variable among females; 800 ppm iodine (either 10 min post-fertilization or after 30 min of water hardening) resulted in nearly complete mortality for eggs from some females and less than 10% for others.

Based upon the above research, achieving 5,000 ppm iodine without killing the eggs might be possible for eyed eggs at pH 8, but unrealistic for freshly fertilized eggs. Alternative chemicals for disinfection may be necessary. Glutaraldehyde was superior to iodine, chloramine-T, and sodium hypochlorite in treatment of plaice (*Pleuronectes platessa)* eggs (Salvesen and Vadstein 1995); concentrations of 400-800 mg/L for 5-10 min was recommended for Atlantic halibut (*Hippoglossus hippoglossus)* eggs, whereas a shorter contact time (2.5 min) was recommended for Turbot (*Scophthalmus maximus)* (Salvesen et al. 1997). For treatment of largemouth bass (*Micropterus salmoides)* eggs, acriflavine (500-700 ppm for 15 min) was the disinfectant recommended by Wright and Snow (1975) over five other disinfectants.

Given the above data, exploration of alternative disinfectants may be necessary for prophylaxis against organisms such as *M. cerebralis* actinospores that may be carried with

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