

Fungal Control Using Hydrogen Peroxide in the Culture of Salmonid Eggs

Andrew J.S. Rubaszek

Parkview Hatchery, Markham, Ontario, Canada

Chemotherapeutants in the Hatchery

The culture of salmonids in a hatchery setting has always been plagued by fungal growth during egg incubation. *Saprolegnia* spp. infect the eggs throughout development; it spreads quickly and egg losses are significant unless it is controlled. *Saprolegnia* settled zoospores and encysted spores stick to the egg surface within 10 min of introduction into incubators and, within 15 min, the encysted spores germinate to form thalli that either spread over or penetrate the chorionic membrane (Rand and Munden 1993). Since *Saprolegnia* is ubiquitous, and hand picking of diseased eggs is very labour-intensive, hatchery personnel have long searched for a chemotherapeutant that is both environmentally friendly and safe for use on eggs.

Although chemical treatments are generally used in hatchery egg culture to control fungal growth, if sufficient manpower is available, or the use of chemical agents is prohibited, hand picking of eggs can produce results similar to those attained by the use of such agents. Visible fungal development is controlled adequately by hand picking of dead eggs (Barnes *et al.* 2000, Barnes *et al.* 2001). But since hand picking is likely only microstatic as it removes only the potential nutrient source for the fungi and leaves behind zoospores, hyphae and bacteria, microbicidal treatments have been typically employed in a hatchery setting.

A variety of chemical agents have been evaluated for their efficacy in controlling fungus. Marking *et al.* (1994) tested 21 chemicals for their effectiveness on *Saprolegnia*. Of this number, seven were found to be effective: Abbott A-73336, amorolfine, formalin, glutaraldehyde, hydrogen peroxide, melaleuca and sodium chloride. Today, only two are in common use: Formaldehyde and hydrogen peroxide. The former, while effective at 1,667 mg/L for 15 minutes daily from fertilization to emergence (Barnes *et al.* 2002) in the control of fungus, is not desirable due to its possible carcinogenicity and effluent concerns. Hydrogen peroxide, on the other hand, is a stable compound that readily decomposes into water and oxygen and is considered safe to the environment. Its antimicrobial properties make it an effective alternative to formaldehyde in the control of fungus on eggs. So much so that considerable recent work has been done to confirm its efficacy and determine an effective, safe treatment regimen (Barnes *et al.* 1998, Arndt *et al.* 2001, Barnes and Gaikowski 2003). Hydrogen peroxide is also being shown to be effective in the treatment of protozoa (Rach and Gaikowski 2001), fungus (Howe *et al.* 1999), and bacterial gill disease (Lumsden *et al.* 1998, Rach *et al.* 2000).

The Parkview Hatchery Operation

Parkview Hatchery (a Community Fisheries Involvement Program (CFIP) facility, sponsored by the Ontario Ministry of Natural Resources (OMNR), and run by volunteers from Metro East Anglers) consists of two buildings located in Markham, Ontario on the Parkview Golf Course. Eggs from Atlantic Salmon, Rainbow Trout, Brown Trout and Pacific Salmon are reared to varying size for stocking in local rivers and streams. Eggs are either collected by electrofishing from local rivers or obtained from OMNR. About 300,000 fish are typically produced and stocked yearly from the operation. Most fish are released as fry, but 10-20,000 are usually reared to fingerlings before release.

The hatchery is supplied by groundwater from adjacent springs. The water is collected in two holding tanks (1000L and 1500L) and then gravity-fed into the buildings. Water temperature is between 5°C and 12°C, depending on season. No treatment of the water is performed and only minor filtering of coarse debris at the holding tank by means of a screen and at each outflow within the buildings by polyester mats occurs.

Building #1 is used for egg incubation and early rearing. It is equipped with 56 Heath vertical incubation chambers arranged in 5 stacks, 3 600 L round tanks, 4 500 L oval tanks and a 500 L raceway. Each tank has its own gravity-fed water supply. Eggs are incubated in this building and reared until either ready for stocking or moved into the other building for rearing to fingerling size. Building #2 contains 5 600 L and one 5700 L tank. There is another 5700 L tank outdoors.

Care of Salmonid Eggs at Parkview

After fertilization, eggs are placed in Heath vertical incubators which are left covered to protect them from direct sunlight. Eggs are handled at a minimum until just prior to eye-up, at which time hand picking of dead and infertile eggs is performed. Picking continues until the alevin emerge. The sac fry are generally left in the incubators until they are free-swimming. At that point, they are placed in early-rearing tanks.

Until 2001, sodium chloride was used to treat eggs to control fungal growth. Success was limited and, due to the labour-intensiveness of using salt, a search was made for alternate treatments. It was decided to use hydrogen peroxide due to its efficacy and safety, rather than compounds such as malachite green and formalin. Since the hatchery discharge flows directly into the local watershed, only an environmentally safe agent could be used.

In 2002, hydrogen peroxide at 500 ppm was used to treat the eggs of Rainbow Trout and Chinook Salmon. The water supply to the Heath incubators was shut off and hydrogen peroxide was added directly to each individual tray to produce an immersion bath of 500 ppm hydrogen peroxide for 15 min. Fungal growth in the treated eggs was much reduced, but two factors influenced a change in methodology for future: 1) adding

a measured amount of peroxide to individual trays was time consuming and 2) there was a concern that the peroxide was not being uniformly mixed by gentle agitation (vigorous stirring during the 70-140 ATU period must be avoided).

In 2003, a new method for treatment was initiated. Using a peristaltic pump, hydrogen peroxide was introduced directly into the water supply line to produce a continuous-flow 15 min bath at 1000 ppm. It was felt that injecting the peroxide upstream directly into the water feed would be time effective, relatively simple and ensure proper mixing of the peroxide. The discussion below details the specifics of this treatment.

Treatment Regimen

Treating eggs daily with 1000 ppm hydrogen peroxide for 15 min has been shown to be effective in the prevention of fungal growth on salmonid eggs (Barnes *et al.* 1998, Howe *et al.* 1999, Arndt *et al.* 2001). However, during the 70-140 ATU period, some mortality due to peroxide treatment has been observed (Barnes and Gaikowski 2003). But withholding treatment during the sensitive period resulted in fungal growth and increased mortality, more so than what was observed in eggs treated with hydrogen peroxide. It is felt that treating eggs continually from fertilization to emergence gives better hatch and lower mortality than by withholding treatment during the sensitive period (Barnes 2003, personal communication). *I.e.*, even though some mortality is expected by treatment during the 70-140 ATU period, it is less than what will be observed due to fungal growth if the eggs are not treated. We therefore decided to perform treatments throughout the entire incubation period.

Ideally, treatments should be performed daily. However, due to limited manpower, we were unable to do so and therefore treatments on Rainbow Trout eggs were performed every two days instead.

Treatment Procedure

Hydrogen peroxide was added directly to the water supply. Using a peristaltic pump, 35% hydrogen peroxide was injected at the holding tank into the pipe feeding the hatchery. The tank is about 30 m from the building, so it was felt that thorough mixing would occur by the time the mixture entered the feeds to the Heath trays.

The supply feed to the building was measured to determine the flow rate. To minimize the quantity of peroxide needed, the flow to the building was reduced to about 20 L/min. An accurate measurement was then made to calculate the amount of peroxide needed. The peristaltic pump was then calibrated to deliver the calculated amount of hydrogen peroxide required to yield a 1000 ppm total concentration in the feed line.

Calculations

The following calculation was used to determine the amount of hydrogen peroxide needed to attain the desired concentration (1000 ppm or .001 Kg/L) in the supply line. Note that the initial calculation is done on the basis of mass.

$$MF = \frac{FCf}{Cs}$$

Where :

F = Flowrate / min (L)

Cs = Concentration of stock solution (percentage)

Cf = Final concentration desired (decimal, Kg / L)

MF = Feedrate by mass (Kg / min)

MF is then converted to a volumetric feedrate (VF):

$$VF = \frac{g / \text{min}}{\text{Specific Gravity}}$$

For example, with a total flow of 18 L/min, using 35% hydrogen peroxide for a concentration of 1000 ppm:

$$MF = \frac{18 \times 0.001}{0.35} = 0.0514 \text{ Kg} / \text{min} = 51.4 \text{ g} / \text{min}$$

Using 1.132 as the specific gravity of 35% hydrogen peroxide at 20°C, converting the above to a volumetric feedrate yields the following:

$$VF = \frac{51.4 \text{ g} / \text{min}}{1.132} = 45.4 \text{ mL} / \text{min}$$

Results

Compared to previous treatments with sodium chloride and individual dosing of trays in a static bath of 500 ppm hydrogen peroxide, the continuous bath with peroxide at 1000 ppm for 15 min served as an effective control for *Saprolegnia*. Quantitative observations were not performed, but anecdotally, the treatments seemed to significantly reduce fungal growth. Previous treatments invariably resulted in continual, although reduced, development and growth of fungus: there were always some clumps or mats of infected

eggs that required considerable hand picking. The present treatment was effective in eliminating the proliferation of fungus to the extent that was observed in the past where matting of diseased eggs was so extensive that it caused clogging of the screens in the trays. No matting and only minimal clumping of eggs was observed, so hand picking was much less time-consuming than in previous seasons. Although the treatment did not totally eliminate the fungus, presumably due to the regimen itself (*i.e.*, every two days rather than daily), the amount of fungus was minimal and the labour required to tend the eggs was much reduced.

To quantify the efficacy of the current treatment regimen, a program is planned for treating Chinook Salmon using controls and rigorous record keeping, with particular attention to condition of eggs at fertilization, number of eggs per incubation chamber and location of tray within the stack.

References

Arndt, R.E., E.J. Wagner and M.D. Routledge 2001. Reducing or withholding Hydrogen Peroxide treatment during a critical stage of Rainbow Trout development: Effects on eyed eggs, hatch, deformities and fungal control. *N. Am. J. Aquacult.* 63:161-166.

Barnes, M. E., D. E. Ewing, R. J. Cordes, and G. L. Young 1998, Observations on hydrogen peroxide control of *Saprolegnia* spp: during rainbow trout egg incubation. *Progressive Fish-Culturist.* 60,67–70.

Barnes, M.E., K. Wintersteen, W.A. Sayler and R.J. Cordes 2000. Use of Formalin during incubation of Rainbow Trout eyed eggs. *N. Am. J. Aquacult.* 62:54-59.

Barnes, M.E., W.A. Sayler and R.J. Cordes 2001. Use of Formalin treatments during incubation of eyed eggs of Brown Trout. *N. Am. J. Aquacult.* 63:333-337.

Barnes, M.E., Sayler, W.A., and R.J. Cordes 2002, Survival of Rainbow Trout sac fry subjected to various Formalin and Hand-picking regimes during rearing in vertical-flow tray incubators. *N. Am. J. Aquacult.* 64:129-135.

Barnes, M.E. and M.P. Gaikowski 2003. Use of Hydrogen Peroxide during incubation of fall Chinook Salmon eggs in vertical-flow incubators. In press.

Howe, G.E., W.H. Gingerich, V.K. Dawson and J.J. Olson 1999. Efficacy of Hydrogen Peroxide for treating *Saprolegniasis* in Channel Catfish. *J. Aquat. Anim. Health* 11:222-230.

Lumsden, J.S., V.E. Ostland and H.W. Ferguson 1998. Use of Hydrogen Peroxide to treat experimentally induced bacterial gill disease in Rainbow Trout. J. Aquat. Anim. Health 10:230-240.

Marking, L. L., J. J. Rach, and T. M. Schreier 1994, Evaluation of antifungal agents for fish culture: Progressive Fish-Culturist. 56:225–231.

Rach, J.J., M.P. Gaikowski and R.T. Ramsay 2000. Efficacy of Hydrogen Peroxide to control mortalities associated with bacterial gill disease infections on hatchery-reared salmonids. J. Aquat. Anim. Health 12:119-127.

Rach, J.J. and M.P. Gaikowski 2001. Hydrogen peroxide a potential broad spectrum therapeutant for treatment of fish diseases. Aquaculture 2001: Book of Abstracts. p. 542.

Rand, T.G. and D. Munden 1993, Involvement of zoospores of *Saprolegnia diclina* (Oomycotina: Saprolegniaceae) in the attachment to and invasion of eggs of brook trout under experimental conditions. J. Aquat. Anim. Health 5:233-239.

Andrew J.S. Rubaszek
Parkview Hatchery
6400 Steeles Avenue East
Markham ON L3S 3J5

andyr@sympatico.ca