

**PROBLEMS ENCOUNTERED  
WHILE USING BAKING SODA SOLUTIONS  
FOR RINSING SALMON EGGS**

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**Abstract**

Pre-rinsing unfertilized eggs with a baking soda solution is an effective way of countering the harmful effects of ruptured egg material on fertilization rates but there are problems when using this procedure in large scale fish culture. With large batches of eggs it is difficult to thoroughly decant the final rinse solution so precise control of the amount of baking soda at fertilization is often impossible. Excess baking soda can result in sperm dilution and reduced fertilization. We found that egg mortality increased linearly from 5 % when the sperm dilution was low, to 24 % when the volume of baking soda was equal to the egg volume at fertilization.

**Introduction**

The presence of even small amounts of material from ruptured eggs interferes with sperm motility and results in low fertilization rates during artificial insemination. Wilcox et al. (1984) found that the contents of just 8 eggs mixed with the ovarian fluid of 3000 eggs reduced sperm motility. In a test group with

1 % broken eggs the mortality was 60 % compared to a control mortality (0 broken eggs) of only 6 %. This is an important effect because eggs can easily be broken by rough handling or by improper use of stripping knives. Rinsing eggs with a solution of isotonic baking soda (13.68 grams/liter) prior to fertilization solves this problem (Wilcox et al. 1984).

This procedure is recommended where broken eggs are suspected or when fertilizing extremely valuable stocks. Although this pre-fertilization rinse is simple and effective, we have encountered some problems during large-scale fish culture operations that can lead to increased mortality.

On the other hand rinsing with baking soda is sometimes performed after fertilization in order to remove excess sperm and blood. This procedure is presumed to reduce fungus growth during incubation. Although this post-fertilization rinse has fewer complications, it does not prevent broken egg material from blocking fertilization.

### **Materials and Methods**

The recommended method for countering the effect of broken eggs is to double rinse the unfertilized eggs in 2.5 times their volume with isotonic baking soda solution ( $\text{NaHCO}_3$ , 13.68 g/l). After decanting the rinse solution, fresh solution, amounting to 25 % of the egg volume, and sperm are added without delay (Wilcox et al. 1984).

We observed that in production-scale fish culture where large egg batches are handled, decanting the baking soda solution in the final rinse was difficult to control. It often resulted in a final volume of solution that was more than 25 % of the egg volume. This greater volume would lead to sperm dilution and possibly reduced fertilization (Billard and Jensen, 1996). To test this possibility, we fertilized batches of eggs containing final volumes of  $\text{NaHCO}_3$  amounting to 0%, 25 %, 50% and 100% of the egg volume. Treatments consisting of a post-fertilization rinse and no rinse (control) were also tested.

Chum salmon from the Big Qualicum Hatchery were used in the study. Eggs from 12 females and milt from 7 males were pooled to reduce gamete differences. There was no signs of broken eggs in the pooled group. Each treatment consisted of approximately 500 eggs (130 ml) and was replicated three times. Using the 25% treatment as an example, the sequence of events was as follows: (a) measure 130 ml of eggs into a container (b) rinse twice with 2.5

times the volume of NaHCO<sub>3</sub> (325 ml) (c) decant completely and add 32.5 ml (25%) of fresh NaHCO<sub>3</sub> (d) fertilize with 2 ml of sperm (e) mix and wait 30 seconds (f) pour into the Heath incubation tray for water activation of the eggs and the start of development. The other treatments were identical except that after the final decant, the volumes of fresh NaHCO<sub>3</sub> added were 0 ml (0%), 65 ml (50%) and 130 ml (100%). The final decant was standardized by fixing a net across the mouth of the egg bucket and inverting for 10 seconds. In the post-fertilization rinse treatment, eggs were fertilized, rinsed twice with NaHCO<sub>3</sub> and poured into the incubator. For the control, eggs and sperm were mixed and poured into the tray after 30 seconds.

Eggs were fertilized on Nov 13, 1998. Incubation trays were observed and the dead eggs were removed and counted on Jan 5 (post-eyed), Jan 8, Jan 25 and March 10, 1999. Live eggs were also counted on Jan 8 so that the exact number of eggs in each treatment was known. Water flow through the Heath stacks was 12 liters/min.

Finally, sperm concentration of the pooled milt was determined using a haemocytometer.

### Results and Discussion

Egg mortality from fertilization to ponding for the six experimental treatments showed significant differences (at the 0.05 level) between treatments ( $F = 14.4$ , critical  $F = 3.1$ ,  $df = 5, 12$ ).

Table 1. Egg mortality (%) for each treatment. Pre-0%, 25%, 50%, 100% indicate the volume of NaHCO<sub>3</sub> at fertilization (as % of egg volume) after the pre-fertilization rinse.

	Control	Post-Fert	Pre-0%	Pre-25%	Pre-50%	Pre-100%
Rep 1	7.2	11.6	4.1	6.8	17.3	24.9
Rep 2	11.4	11.2	4.5	8.7	11.4	19.9
Rep 3	7.2	5.1	6.3	7.3	8.2	27.4
<b>Mean</b>	<b>8.60</b>	<b>9.33</b>	<b>4.94</b>	<b>7.57</b>	<b>12.33</b>	<b>24.08</b>

The treatment with the greatest volume of NaHCO<sub>3</sub> at fertilization (Rinse 100%) had the highest mortality. Most of the dead eggs were removed on Jan 5 and Jan 8, 1999 and showed no signs of development. Hence, for these eggs it is

presumed that sperm were too diluted in concentration and that fertilization did not occur.

Regression analysis showed that there was a significant relationship between egg mortality (Y) and the amount of baking soda at fertilization (X) (Fig. 1). The least squares trend line in Figure 1 is given by:  $Y = 3.6361 + 0.1964X$  ( $r^2 = 0.875$ ).

Figure 1 shows that failure to control the final volume of baking soda at fertilization can lead to increased mortality.

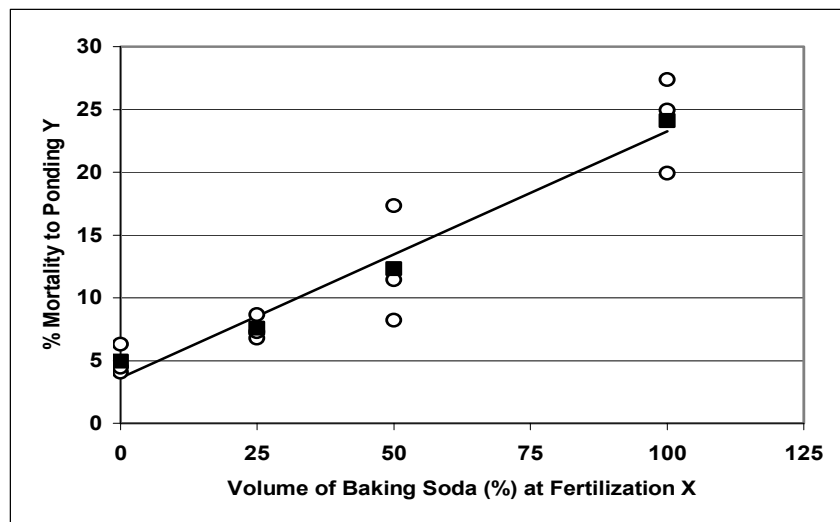


Figure 1. Egg mortality (%) Y versus volume of baking soda at fertilization (as % of egg volume) X. Replicates, treatment means (solid squares) and the trend line are also shown. The equation for the linear relationship is  $Y = 3.6361 + 0.1964 X$ ;  $r^2=0.875$ ,  $F=70.2$  (critical  $F=4.96$ ,  $df=1,10$  at 0.05).

To see how this could happen during large scale fish culture consider the following example where the pre-fertilization rinse procedure is being used on a 1 liter batch of eggs. During the final rinse 2.5 liters of  $\text{NaHCO}_3$  is used. If the final decant is performed simply by tipping the bucket on the floor, it is conceivable that a considerable volume of  $\text{NaHCO}_3$  is left in the egg bucket when the final 0.25 liters of fresh  $\text{NaHCO}_3$  is added just prior to fertilization. If

2 liters was removed in the final decant so that 0.5 liters was left in the egg mass, the final volume after addition of 0.25 liters of fresh NaHCO<sub>3</sub> would be 0.75 liters (75%). With the protocols used in this experiment (2 ml milt per 500 eggs, 30 second contact time), this procedure would cause significant increase mortality (Fig. 1). Using baking soda to counter the effects of broken eggs requires careful decanting and accurate volume measurements so that the final volume of NaHCO<sub>3</sub> is no more than 25% of the egg volume.

The sperm concentration from the pooled sample was found to be  $26 \times 10^9$  per ml. Hence, egg mortality, when plotted against sperm concentration (Fig. 2) in the various final rinse volumes resulted in the following curvilinear relationship:  $Y = 5.1993811 + 1.5344362e+14/X^{1.5}$  ( $r^2 = 0.8927$ ). This translates to 16 million sperm per egg for the treatments reported herein, which is greater than the 0.5 to 1.0 million sperm per egg concentration suggested by Billard (1992) as being adequate for successful fertilization. Hence, other factors such as sperm concentration, temperature, and contact time prior to water activation likely interact to affect fertilization success. Given the conditions in our tests, egg fertilization was noticeably reduced in all rinse treatments compared to the control.

Another potential problem with this method can occur with the warming of the baking soda solution. Large volumes must be prepared and stored over several hours during egg takes. Depending on the initial water temperature and air temperature the solution can warm to lethal levels. On warm days it may be necessary to store the baking soda in a flowing water bath. Temperature should be monitored and the solution discarded if it is significantly warmer than the incubation water supply.

This procedure should be tested on a production scale before being fully implemented at a hatchery. Practical procedures must be established for: (a) preparing and handling large volumes of baking soda (b) decanting the rinse solution (c) control of the final NaHCO<sub>3</sub> volume at fertilization. These new procedures will require more manpower and will increase handling times. To avoid problems and reap the benefits of this very useful technique, careful planning and production scale testing are required.

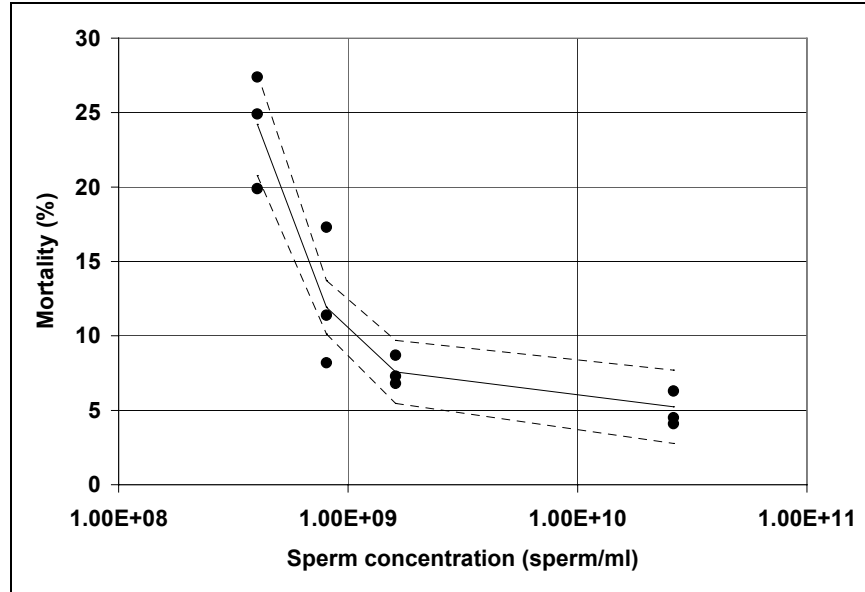


Figure 2. Egg mortality (%) versus sperm concentration (sperm/ml). The equation for the curvilinear relationship is  $y = 5.199381 + 1.534436e+14/x^{1.5}$ ;  $r^2=0.8927$ ,  $F=83.2$  (critical  $F=4.96$ ,  $df=1,10$  at 0.05). The replicates (solid circles), the curvilinear line (solid line) and 95% confidence limits (dotted lines) are shown.

## References

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