

**A MASS VACCINATION TECHNIQUE THAT PROVIDES
GOOD PROTECTION AGAINST VIBRIOSIS
IN CHINOOK SALMON (*Oncorhynchus tshawytscha*) SMOLTS**

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Abstract: A very simple method for simultaneously vaccinating large numbers of chinook salmon (*Oncorhynchus tshawytscha*) smolts was compared to the conventional dip immersion vaccination method. While not performing as well as the dip method (90% relative percent survival [RPS] over unvaccinated controls), the mass vaccination method described gives a good level of protection (65-90% RPS). Since our fish reside in sea cages or a very short time (1-3 wks) before being released, and are only occasionally exposed to *Vibrio* in the ocean if water temperatures are unseasonably high, we felt that the level of protection is adequate for our purposes and have continued to use this method for the last 8 years with 100% success. The mass vaccination method is also about 10 times less costly in manpower than the dip immersion method, making the vaccination procedure an insignificant cost in the hatchery operation.

Introduction

At Salmonid Enhancement Program (SEP) facilities, chinook salmon (*Oncorhynchus tshawytscha*) are vaccinated against vibriosis prior to transfer to sea cages for short-term acclimation rearing. The most common procedure world-wide for vaccinating fish is to immerse a netful of fish into a 1:10 (bacterin [killed bacteria] to water) solution of commercial vaccine for 20-30 s (Eddy, 1988). Each liter of bacterin solution can adequately vaccinate about 100 kg of fish, so usually a 100 L container is prepared with 10 L of bacterin solution and a series of netfuls of 5-10 kg of fish each are dipped into the diluted vaccine one after the other. The vaccine/water mix is usually aerated but the water quality degrades rapidly with the addition of slime and faeces, so that the last group in a series is taking a bath in water that 200 other groups have already bathed in. This netting and handling is obviously stressful to the fish and is also manpower intensive, requiring hours of heavy labor, and carries the associated costs. Although the first trials of a 'bath' vaccination method, using a dilution

rate of 1:200, bacterin:water for a 2 hr bath, provided almost 100% protection from vibriosis, although it was only a lab-scale test (Egidius and Andersen, 1979), later application of *in situ* bath vaccination have used dilution rates in the 1:1000 to 1:5000 range, extending for several hours. This method, where fish are exposed to a more dilute solution of bacterin for a long period, requires less fish handling but can cause stress and poor bacterin uptake from long term exposure to stagnant water loaded with suspended matter, even if oxygen levels are maintained with artificial aeration or oxygen injection. This method has come out of favor because of the lower performance compared to dip immersion.

The purpose of this study was to try to determine the easiest-to-apply combination of dilution of bacterin and duration of exposure for mass vaccination to get a reasonable level of protection in production groups of fish. This paper reports on tests conducted over two years at Tenderfoot Creek Hatchery.

Methods

The basic technique described here involves applying the vaccine to the fish in their rearing containers, rather than removing them from their containers and taking them to the vaccine. The first step in the mass vaccination method is to gently increase the density of fish in their container to get the target dilution rate. The fish were not fed for two days and the raceways were brushed clean (although this work is minimal in our self-cleaning, baffled raceways) to reduce the amount of suspended material in the water. To vaccinate fish in their raceway, we first crowd the fish into the downstream quarter of the container, into which has already been placed a grid of ceramic air stones hooked to a supply of pure oxygen. We find that stress in the fish is reduced if we provide a cover (a floating tarpaulin) over this area of the raceway prior to and during the crowding operation. In our case, this procedure puts 200,000 3-5 g fry into an area 3 m long, 2.5 m wide and 2 m deep, giving a density of 40-66 kg/m³. We then remove stoplogs one by one to drop the water level in the raceway to the target depth. To find the depth of water in this area to achieve the target dilution, we use the formula:

$$Z = \frac{V_{Dil} * n * w}{V_{App} * L * W}$$

where: Z = required depth (mm), V_{dil} = target dilution rate in volume (L) of vaccine to one liter of water, n = number of fish, w = weight of each fish (kg), V_{app} is the recommended application rate for the vaccine (kg fish/L vaccine), L and W are the length and width of the crowded area (m). For a 500:1 vaccine:water dilution in our situation, the water depth would be 0.4 m. When the water level has reached the correct depth, we turn on the oxygen and turn off the water supply.

When the water stops overflowing the stoplogs at the downstream end of the raceway, we replace the stoplogs to the normal operating level and, after removing the cover, spray the prescribed amount of commercial bivalent (a mixture of *Vibrio anguillarum* and *Vibrio ordellii* bacterins) vaccine over the surface of the water only in the section that contains fish. The amount of vaccine we apply (according to the manufacturer's specifications) and the water volume determine the dilution rate. We then turn on the flow of fresh water. Since the water enters at the opposite end of the raceway from where the fish are crowded, the water level rises gently and slowly infiltrates the area where

the fish are located, improving the water quality as the raceway fills up. In our current procedure, it takes about 30 minutes to refill the raceway to where water starts spilling over the stoplogs at the downstream end. Various dilutions were also tested on a smaller scale using 100 L plastic tubs and adjusting the number of fish to attain equivalent densities and bacterin/fish dilution rates as would be used in the raceways.

We removed samples of fish from the raceways and tubs after intervals varying from 0.5-30 min and placed them in clean flowing water. This allowed us to evaluate the effectiveness of leaving the fish in the vaccine for short or long periods, which we call the duration of the test. To test the protection afforded by the different vaccine dilutions and durations, we reared groups of 200 fish from each treatment, and a crowded-but-not-vaccinated control group, for 7 wks at 7°C to allow immunity to develop. We then conducted a disease challenge by exposing four replicates of 50 fish each to two levels of live *V. anguillarum*; 6×10^3 and 6×10^6 cells/ml for 3 min. The fish were then reared at 10°C, recording mortalities until they tapered off, which was usually complete after two weeks.

According to Amend (1981), duplicate groups of fish should be challenged at two levels with at least 25 fish in each of the four groups. At least 60% of the controls should be killed by the challenge. Effective vaccines should limit infection in the treatment groups to less than 24%. Differences between vaccine treatments should be at least 20% to be considered statistically significant. These conditions were all met in this experiment.

The relative percent survival (RPS) was calculated for the different dilution and duration rates using the formula: (MT = the treatment and Mc = the control mortalities).

$$RPS = (1 - (\frac{M_T}{M_C})) * 100$$

This is an index of protection from the live bacteria that uses the mortality in the control group as a baseline from which to evaluate the performance of the other treatments.

Results and Discussion

Consistent with the findings of many other workers (Hjeltnes et al. 1989), the highest concentration of bacterin (1: 10 dip treatment) had the highest relative percent survival (89-92% RPS) compared to unvaccinated controls (Figure 1). However, dilutions of 1:100, 1:250 and 1:500 all had RPS of over 80%, which indicates very effective vaccination. We assume that the level of protection required for the short term that SEP fish are reared in sea cages is not as great as that required for fish grown to market in sea cages. Our fish are normally released from the sea cages in mid May, although some groups have been released as late as the end of June. Water temperatures are usually too cool for *Vibrio*, but one year with a warm Spring, an unvaccinated group of fish still in their sea cages in mid June were exposed to water temperatures over 20°C and contracted a severe case of vibriosis that virtually wiped out the whole group in about 3 days. Because of the rare nature of exposure to live *Vibrio* during sea cage rearing, we feel that this lower level of protection may be adequate for the needs of our program.

Contrary to expectations, protection decreased with increased exposure time (Figure 2). Most studies

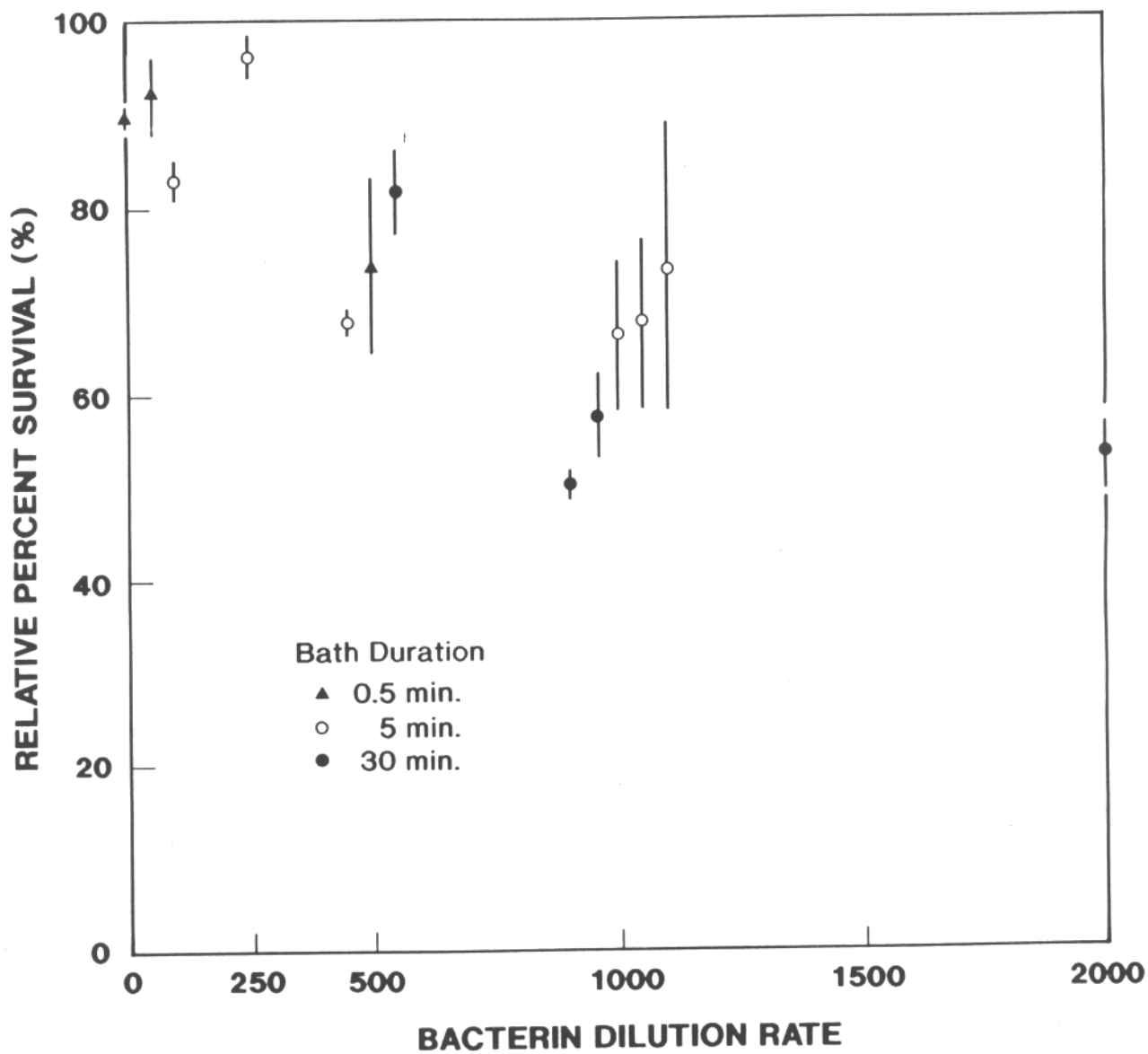


Figure 1. The effect of bacterin dilution rate on performance at different bath dilutions.

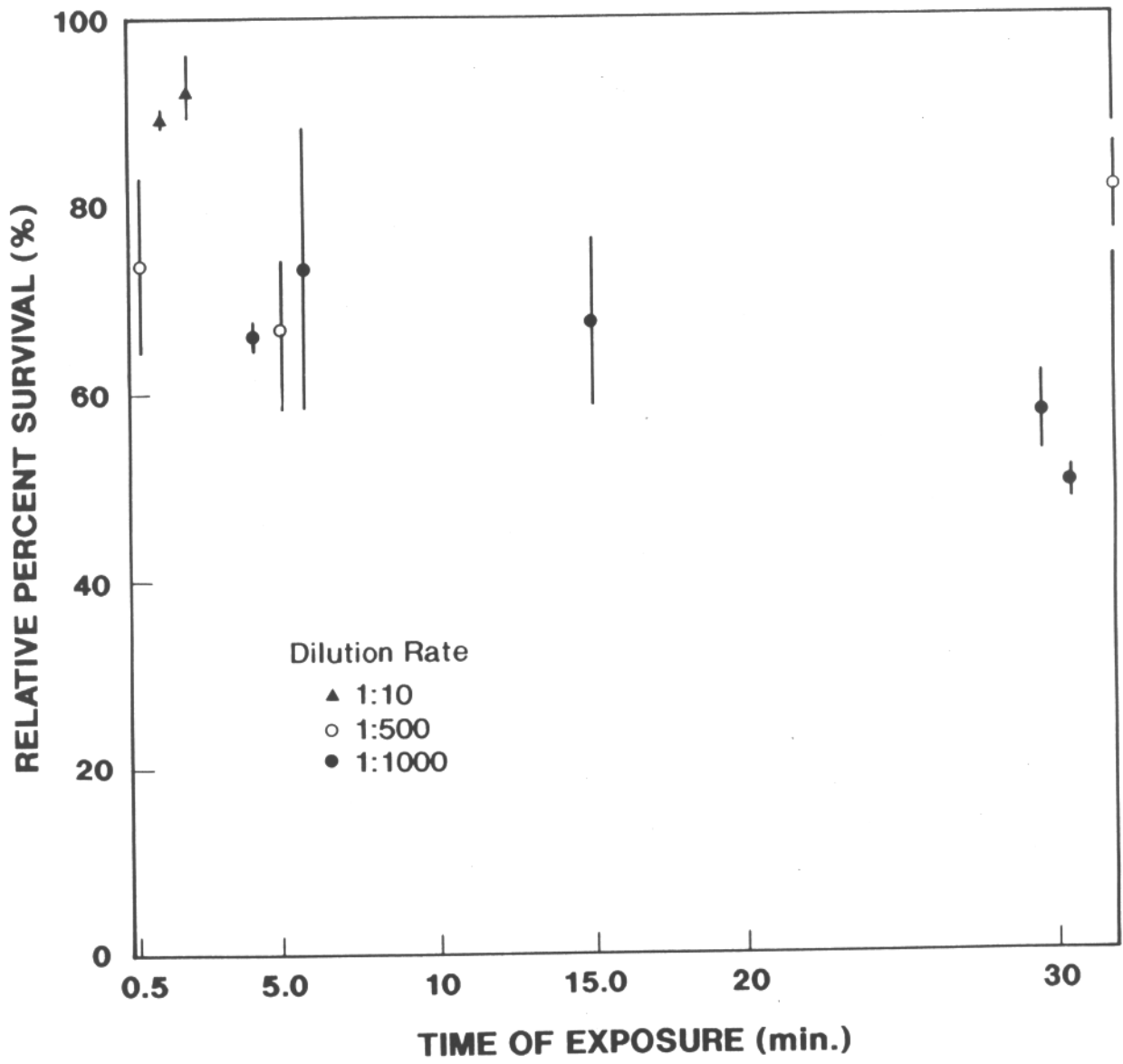


Figure 2. The effect of duration of exposure on bacterin performance at various dilution rates.

and manufacturer's recommendations suggest that increased exposure time will improve uptake of bacterin, leading to greater protection. However, those same studies show that bath vaccination always performs worse than dip immersion vaccination, whereas with our method, some of our mass vaccinations were as effective as the dip immersions. The decrease shown in this study was probably due to the cumulated stress of high crowding in stagnant water during the treatment. During the tests, we let the fish sit in stagnant water for the 30 min of the test duration, but subsequently, we have changed our technique to allowing only 30 s of stagnancy, after which we fill up the raceway as described above. Checks of immunocompetence with this technique have shown a very high level of protection.

The best way to take advantage of the lower stress on the fish that a mass vaccination affords seems to be to apply the bacterin as quickly as possible to a concentrated group of fish, followed by a rapid return to high quality water conditions.

As far as cost is concerned, it used to take 3 hrs for a crew of four to vaccinate a raceway holding 200,000 fish using the dip immersion method. Using this mass vaccination method takes a crew of two about ½ hr per raceway. With eight raceways to vaccinate, the difference between the methods is 96 man-hours versus 8 man-hours to vaccinate our entire production. This considerable cost savings is contrary to the findings of Lillehaug (1989), who used a very long bath method applied in an inefficient manner, resulting in a conclusion that the dip immersion was less expensive.

We will continue to use the mass vaccination method because of the advantages in cost and time and the reduced stress to the fish. If we were concerned with improving the immunity of our fish, we would consider a revaccination using the same simple method, to boost the protection against *Vibrio*.

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