

**THERMAL MARKING OF ALEVINS TO ENABLE  
IDENTIFICATION OF HATCHERY STOCKS**

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**Abstract:** The contribution of hatchery releases to returning adult chinook salmon in the Chilliwack River has been assessed using two independent methods. Fish that swim in to the Chilliwack River Hatchery are counted and the count is adjusted by the proportion of marked versus unmarked fish that were released as smolts to estimate the enhanced contribution. The total number of fish that spawn in the river is estimated from dead pitch sampling expanded to account for only 8-12% of total return being accessible to the dead pitch, estimated from a previous Peterson tag-recapture study. The number of tagged fish in the dead pitch is expanded by the tagged-untagged ratio of the released smolts. These estimates do not account for differential survival between tagged, untagged and wild spawned fish, differential return locale preferences of wild versus hatchery origin returning adults and the potential errors in the total wild spawner return estimates. By adjusting incubation temperatures on an evenly timed schedule, we induced the formation of dark bands on the otoliths of alevins and were thus able to mark all of the hatchery-origin fish with no stress to the fish and at negligible cost. When these fish return in 3-5 years, we will be able to sample the wild and hatchery returns to obtain an independent and unbiased reading of the ratio between wild and hatchery-origin fish, and therefore gain a better understanding of the contribution that the hatchery makes to the spawning population.

**Introduction**

The Chilliwack River fall chinook run began as a transplant in 1981 of 675,000 eggs from the Harrison River run collected at the Chehalis River Hatchery. Transplants ceased in 1984, when

sufficient adults returned to the Chilliwack River Hatchery for fall run broodstock.

This run is assessed annually with a coded wire tag program, in which recoveries take place in the various commercial, sport and native Indian fisheries. The methods used are described by Kuhn et al (1988) and come under the auspices of the Mark Recovery Program (MRP), an international cooperative accounting of salmon production from hatcheries in the eastern Pacific. All coded-wire-tagged fish have their adipose fin removed so that marked fish can easily be identified as adults. The escapement to the river for natural spawning and to the hatchery for broodstock are also assessed.

Natural spawning escapement sampling consists of a dead pitch to estimate both total escapement and the proportion of fish of hatchery origin. The total escapement estimate is based on a comprehensive Peterson tag-recapture program (Ricker, 1981) conducted in 1986 which estimated that approximately 12% of the total returns to the river were accessible to the dead pitch sampling. Dead pitch recoveries are expanded by this factor to get the total estimated escapement. The hatchery-origin contribution originates from the expansion of coded wire tagged recoveries from the MRP. In some years, the enhanced hatchery-origin has accounted for 100% of the natural spawners, while in others it has ranged from 54 to 86% (Figure 1.).

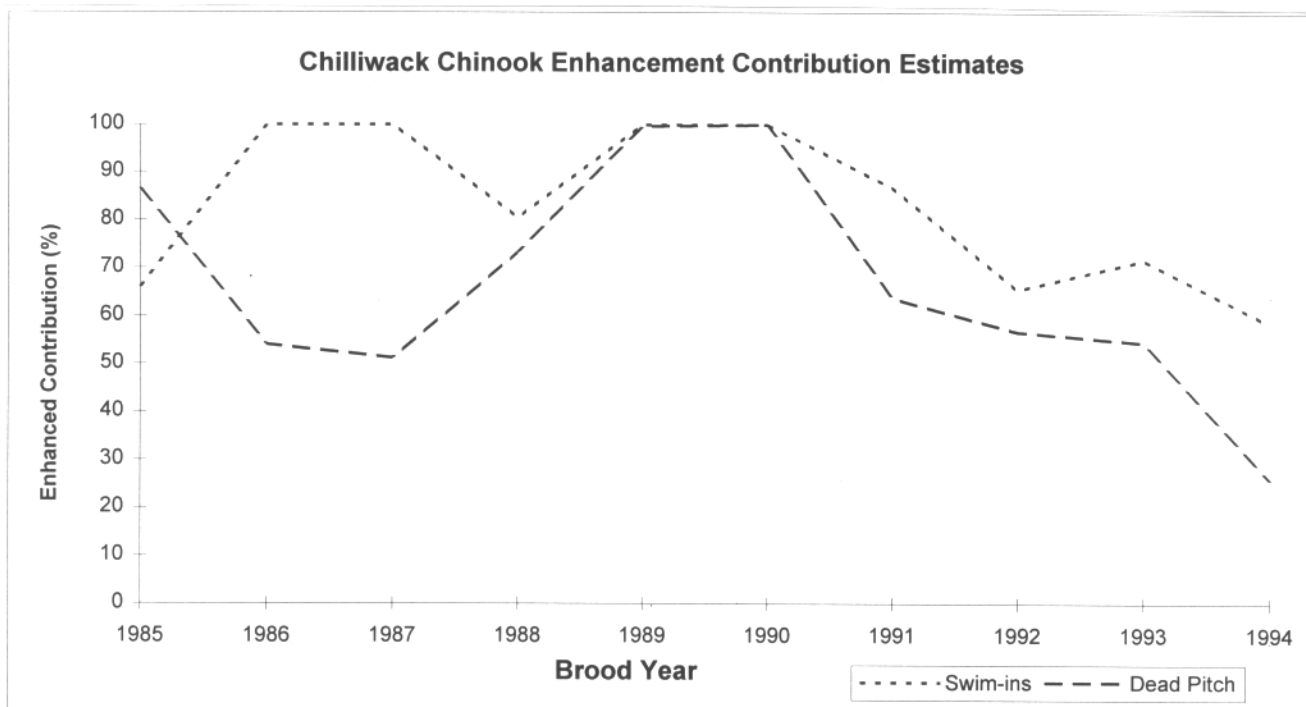


Figure 1. Results of different methods of estimating hatchery and wild-spawn contributions to Chilliwack River and Hatchery returns.

The hatchery-origin proportion of the fish that swim in to the hatchery is estimated by expanding the number of marked fish (no adipose fin) counted by the ratio of marked-to-unmarked fish released from the hatchery in the applicable brood years. All of the marked fish are eventually identified as to which release group they belong by reading the coded-wire tag.

Considering these data, it would appear that naturally spawning fish, although out-produced by the hatchery, are contributing to the escapement and that the transplant is now naturally propagating. However, difficult escapement sampling conditions have often rendered the estimated proportion

of hatchery origin questionable. In any event, there was often a large discrepancy in the results from the two methods of estimating the hatchery and wild-spawn contributions.

In order to further examine the natural versus hatchery contributions, 1995 brood year hatchery fish were marked with a thermal otolith mark, as will be some subsequent years. Escapement will then be sampled, starting in 1998, for otolith marked fish so that hatchery and wild contribution can be independently estimated. Because every hatchery-origin fish will have an otolith mark, relatively small sub-samples of the wild and hatchery-return escapement should give statistically accurate estimates of their respective contributions to the total escapement.

## Methods

The thermal marking procedure was based on the work of Brothers (1985) and Volk (1994) and refined into a stepwise procedure by Hoyseth (1995). The basic procedure is to decrease the incubation temperature by at least 2°C for 24 hr and then return to the original temperature for at least another 24 hr. A sequence of temperature drops and increases causes an increase in the amount of calcium laid down in bone growth rings during the lower temperature phase, resulting in alternating dark and light rings visible at the margin (which later are found near the center) of the otolith.

To get a consistent mark for a stock of fish, the thermal marks should be applied at approximately the same stage of egg/alevin development, so that the mark will be laid down in the same area of the otolith for each fish. Most of the chinook salmon eggs at the Chilliwack hatchery are taken over a period of a month, so the eggs were grouped into three different groups, approximately 10 days apart in their timing. The alevins were marked at 10 day intervals so that they would be close to the same stage of development during the marking procedure (Figure 2.).

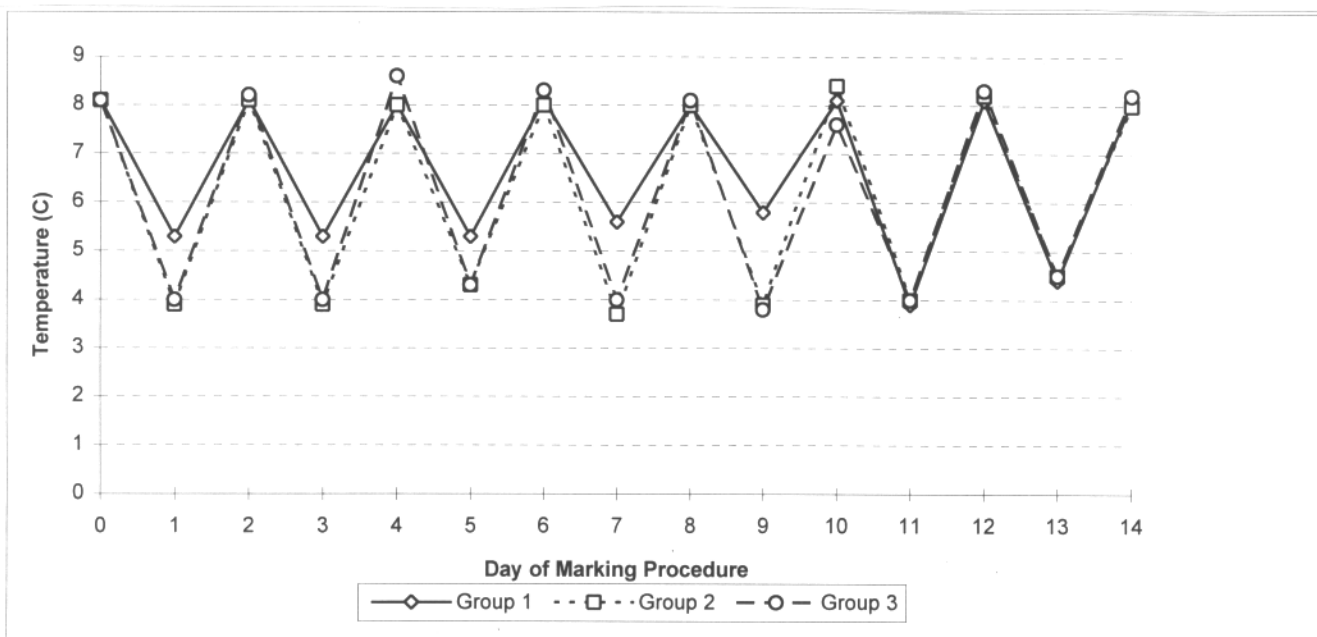


Figure 2. The temperature profile used to induce a thermal mark on chinook salmon alevins at the Chilliwack River Hatchery for the 1995 brood year. The chart shows the daily mean temperature for each day of the marking procedure. It actually took only one hour to adjust the temperatures, not a gradual adjustment as might be interpreted from the chart.

The temperature drops in the incubation water at the Chilliwack Hatchery were accomplished by switching from a mixture of well and ambient river water to only ambient river water, and then switching back to a mixture. The temperature pattern used to mark the alevins shows that at least the minimum drop of 2°C was attained with each change of water supply.

Samples of the fish were taken at the alevin and rearing (just prior to release at 6 g) stages to check that a valid mark had been imprinted on the otoliths. The fish were fixed in 90% denatured alcohol and shipped to the reading laboratory. Otoliths can be removed from fresh, frozen or preserved specimens. Preservation in 80-100% alcohol is considered very important because formalin seems to disintegrate otoliths. Sagittal otoliths were removed from the ear canals of the fish using a dissecting microscope, cleaned and affixed to a microscope slide with thermoplastic cement, melted on a hotplate. These small otoliths from alevins and juveniles were mounted sulcus side up, while otoliths from adults are mounted sulcus down. The otoliths were then wet-ground to mid-plane and polished using geological lapping film (a special type of fine sandpaper) of decreasing grit size (approx. 60 $\mu$  to 1 $\mu$ ). Larger otoliths are ground with coarser paper (approx. 300 grit to 600 grit) on a machine to mid-plane, flipped over, and ground on the other side to mid-plane. They are then polished for examination.

The otoliths were examined under a compound microscope (100X to 400X) for the presence of thermal marks. Careful measurements were made using an eyepiece micrometer and photographs were taken of each otolith. Distances from the centre of the otolith (core) to the start of the basemark, basemark width, and focus to accessory mark were taken for each sample. The measurements were taken in the same quadrant of the otolith and abnormalities were noted. These procedures are meant to give a thorough profile of the thermal marks in the juveniles, so that they can be correlated to the marks found in returning adults, wherever they are captured.

## Results

This paper only reports on the first phase of this experiment, that of placing and verifying the thermal marks on the alevins and juvenile fish before their release from the hatchery. A very good mark was obtained using the method described above. We had some equipment problems that did not allow us time to produce photographs of the 7-banded otoliths from this experiment, but we have included photographs of a 4-band otolith produced with the same method (Figure 3).

When the adult salmon return from the ocean, they will be sampled for otoliths by taking random samples from the populations swimming into the Chilliwack River Hatchery and those spawning in the Chilliwack River. Since all of the hatchery-origin fish will have a thermal mark, the escapement sampling program will only have to sample a small proportion of the returning fish to obtain a statistically valid estimate of the enhanced contribution. This will give an independent, and more accurate, estimate of the proportion of the fish that are hatchery or wild-spawner origin.

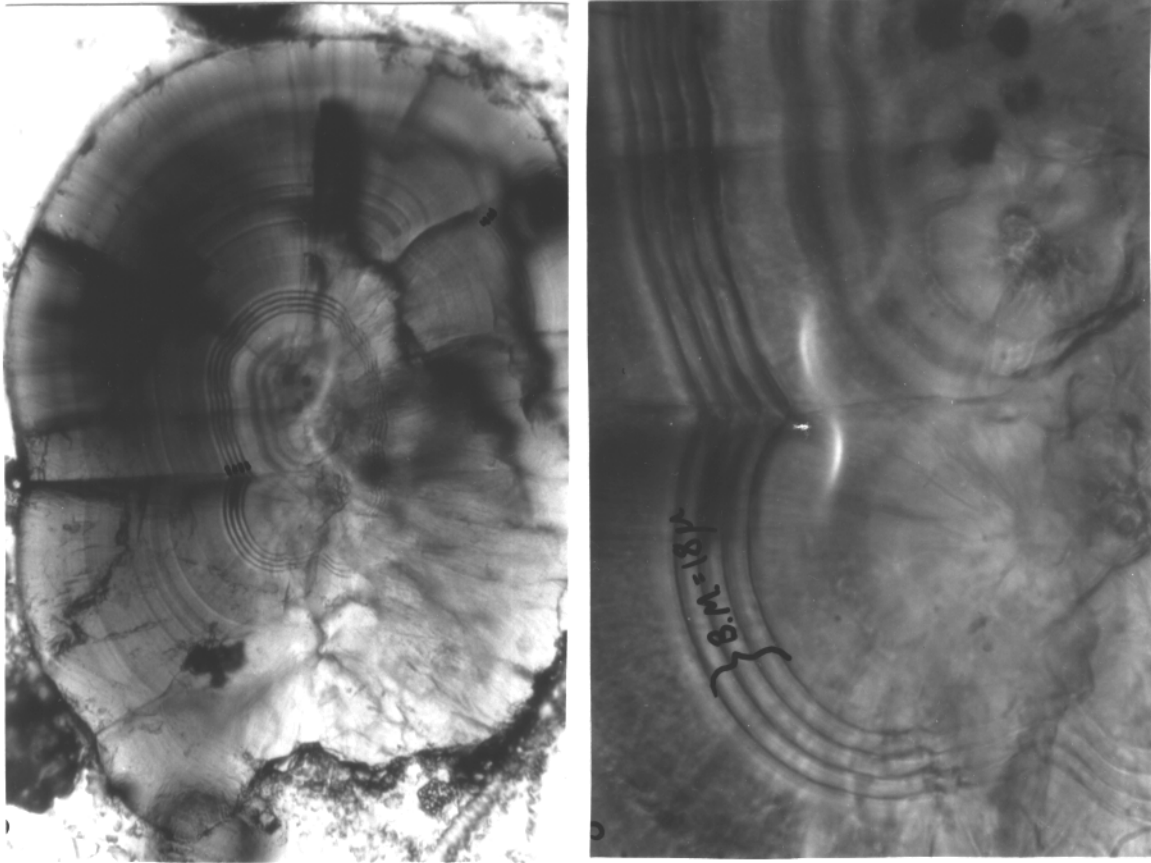


Figure 3. Photographs of a 4-band thermal mark from a chinook salmon at 200X (left) and 400X (right), showing the even distribution from daily temperature changes for four days.

### Acknowledgements

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